UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Service
Entomology Research Division

# Proceedings

of

# Planning and Training Conference

for

# Insect Nutrition and Rearing

## Sheraton-Elms Hotel
## Excelsior Springs, Missouri

March 4-8, 1963

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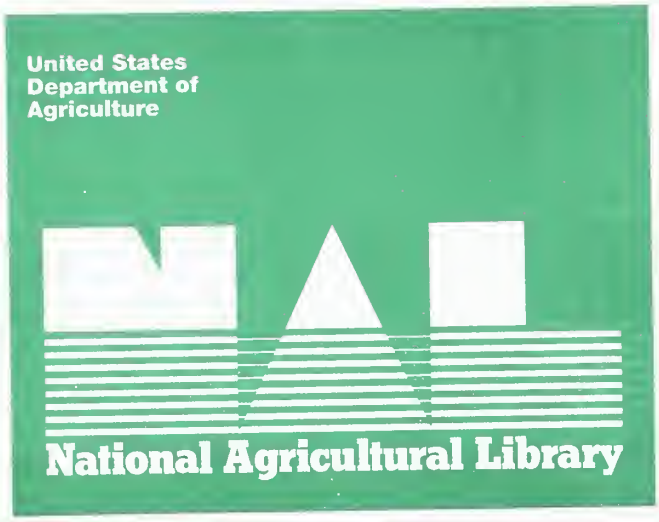
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# Preface

The ability to colonize insects successfully in the laboratory is one of the most vital requisites to progress in many phases of entomological research. Success in this basic aspect of entomology is of equal importance whether destructive or useful insects are being investigated. Aside from the importance of laboratory insect colonies to facilitate research on many aspects of investigations that are desirable on the species involved, the eventual success of several approaches to insect control now receiving increased attention will depend on the progress that can be made in the development of mass rearing methods.

Many important advances in research on insect nutrition, insect colonization techniques, and on mass production methods have been made during recent years. Success has been particularly outstanding in the field of nutrition. Some difficulties have been experienced in developing colonization techniques and mass rearing methods for certain species but in general tremendous progress has been made in these fields also. For many species no concerted effort at developing rearing methods has yet been made.

The purpose of the Insect Nutrition and Rearing Conference was to bring together most of the Entomology Research Division personnel now actively engaged in research on insect nutrition and rearing and those who plan to initiate or direct such research in the near future so that they could exchange information and more intelligently plan their work programs.

These proceedings consist of summaries of research accomplished, or underway which were presented at the conference. Most of these are in the forms submitted by the authors. The summaries are arranged in three major groupings as presented during the program--Basic Research on Insect Nutrition, Panel Discussions on Diets and Techniques for Rearing Insects for Experimental Purposes, and Mass Rearing for Control and Eradication Programs.

Many excellent films and picture slides, illustrating rearing techniques used in the Division were also presented. After these have been assembled and edited, they will be made available to all field stations, cooperators, and other interested personnel on a loan basis. It is hoped that in this way, all who could not attend the Conference, can benefit from the material presented on this important subject.

The Program Committee wishes to thank Drs. P. S. Callahan, Tifton, Georgia, and B. W. George, Brookings, South Dakota, for furnishing the photographs used in these proceedings. They are also grateful to Mrs. Estella Hanson, Secretary, Grain and Forage Insects Research Branch, for collecting, organizing, and typing the abstracts presented.

# Introductory Remarks

E. F. Knipling, Director
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The ability to rear insects is basic to rapid progress in entomological research. This is true for many reasons. First of all just being able to maintain the insect colony the year round gives the research workers an opportunity to work on his problem the year round.

I remember working on disease-carrying insects at Orlando, Florida, during World War II. I think the reason that the Orlando Laboratory succeeded in accomplishing its objective so quickly was that primary attention was given to the development of insect rearing methods for several of the insects we were working with. By developing a method of colonizing the body louse, for example, we were able to develop an excellent insecticide for its control in less than 6 months. If we had depended on natural infestations, it might have taken years to develop a good treatment. The same thing applies to every other insect we worked with, mosquitoes, flies, ticks, etc. In many cases the rate of progress in solving an insect problem depends on the development of good methods for maintaining a colony.

Insect nutrition is a part of this conference. This is an area of research which has not received enough emphasis. This type of research can help us a great deal in solving the many problems associated with insect rearing. It may be the key to developing methods of insect control. A good example is the nutrition studies on the fruit fly conducted in Hawaii, which led to protein hydrolysate bait sprays, as well as to improved methods of mass rearing the various fruit flies. Research on varietal resistance to insects in crops cannot be carried out efficiently unless we can maintain colonies of insects in the laboratory or unless we can maintain colonies of insects on the crop itself. Successful culturing or rearing insects is basic to entomological research of all kinds--insect physiology, insect behavior, etc.

The Division is becoming more and more interested in insect pathogens. The ability to mass produce and use certain microbiological agents may depend on our ability to mass produce the insect host. Excellent progress in mass producing the cabbage looper and the production of the looper virus at Brownsville is an example. The mass production of parasites and predators for release to achieve immediate and direct control may also be feasible for some insects.

The Division has also become more and more interested in insect attractants. I would say that in our research on insect sex attractants, the mass production of the insect may be necessary in order to obtain sufficient material for chemists to identify and synthesize the attractant.

Two years ago we had a conference on insect sterility. If we are going to use this method, it will be essential that we rear insects in quantity. But the cost of rearing insects that will prove practical will depend on many circumstances. If it is difficult and costly to rear some insects, we are inclined to say the sterile male method is not feasible. Sterile male insects may be useful in some situations if it costs $1.00 each to produce them. However, I believe that in general we can expect to produce insects at a lower cost than we first expect. Costs for rearing screw-worms amount to about $1.00 per 1,000 at first. Now as a result of research at Kerrville and Mission, this cost is about 25¢ per 1,000 or $250 per million. Remarkable progress has been made in rearing fruit flies. I believe our laboratories at Hawaii and Mexico City could develop mass production methods for fruit flies at costs substantially less than $200 per million.

I realize that with many insects we may not be able to achieve highly efficient methods on a small scale but when basic methods are turned over to engineers, they can help reduce production costs when converted on a mass production scale.

We should not overlook the possibility of rearing insects on their natural host. For example, the Oxford, North Carolina, Laboratory has had good success in growing the tobacco hornworm in large numbers on tobacco in cages and in the laboratory. I have given a lot of thought to the boll weevil and the pink bollworm. Laboratory methods should be developed but it may not be absolutely essential to mass produce boll weevils and pink bollworms in the laboratory on synthetic diets. If necessary, we may be able to do this on cotton plants growing in the field.

The Japanese beetle is an insect that has only one generation per year. If we gave a lot of thought and effort to possible rearing techniques for this insect, we could probably rear Japanese beetles in their natural environment in quantities that would make it practical to use sterile males for eradicating insipient infestations or for eliminating low level populations that often survive after applying chemicals in eradication programs.

We should not overlook the possibility of rearing some insects in large cages on their natural hosts. Dr. McKibben, Director of the Agricultural Engineering Research Division, made some estimates for me on the approximate cost of cage construction for the rearing of tsetse flies in Africa. He gave me estimates of what it would cost to construct the cheapest cage possible to cover 10 acres. These involved costs for wire, posts, screen materials, etc. Such a cage would be

similar to cages used for growing shade tobacco. The cheapest cage would cost $6,000 -$7,000. To use the same type of frame work and use plastic screen, the cost might be $20,000-$40,000. Even this higher cost figure is not necessarily prohibitive for some insects when you prorate the cost over a 3 or 4 year period. The cost that you can afford to pay for rearing insects will depend on the circumstances and the nature of your objective.

# Development of Defined Diets for Phytophagous Insects

Erma S. Vanderzant, Biochemist
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Plants contain many substances that are not used as nutrients by insects. The composition of plants also may vary with climate, nutrition, day length, and many other factors. Diets of known composition can be easily reproduced, the essential nutrients for insects can be determined, and the effects on insect development of substances added to the diets can be studied.

Several terms have been used to describe diets that are different from the natural diets of insects. The most general term is artificial, describing any diet that is not the natural food of the insect or contains natural food modified in some manner. Synthetic is an ambiguous term. To some workers it means that a diet was compounded from pure chemicals; to others it only means artificial. Purified is a term that should be applied to certain nutrients such as casein. In the broadest sense a defined diet is one that contains chemically pure nutrients and one or more highly purified natural products, such as casein. Eventually all nutrients must be replaced by simple compounds. Hence, amino acids are substituted for protein, and fatty acids for fat. For each test the experimental limits for the diet should be explained.

A good rearing diet should produce a yield of adults from larvae of 70% or more. Size and rate of development of the insect should be similar to those in nature. The adults should mate and lay viable eggs. In our laboratory, good defined diets have been developed for the pink bollworm and both larva and adult of the boll weevil.

With diets composed of purified substances many problems arise. Some dietary constituents may contain trace substances in amounts sufficient to mask or interfere with the effect on growth of another nutrient. Even simple chemical compounds such as the L-amino acids may contain impurities that affect growth. An insect may reject a diet and die because some dietary substance does not taste good. The substance may be a feeding deterrent rather than a toxin. Omission of a nutrient also may prevent feeding. Growth may be inhibited because of an improper balance of nutrients in the diet rather than the ionic character of the diet. The importance of nutrients carried through the egg often is overlooked. The effect on growth of nutrients stored in the egg depends on the ratio of size of egg to adult. The rearing of an insect for several generations on defined diets should expose any dietary nutritional deficiencies.

# Food Plant Preferences in Phytophagous Insects

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The food plant range and preferences of the tobacco hornworm, Protoparce sexta, is given as an example of an oligophagous insect. In this species, plant preferences are displayed by both the adults and the young, but initial choice of food plants for the young is largely a matter of parental concern. The adults utilize chemical cues or stimuli emanating or resident in the plants for (1) orienting to the plant, and (2) oviposition. The stimuli are an attractant or attractants and an oviposition stimulus. Both of these stimuli are found in solanaceous plants, the normal food plants of the larvae. The attractant is steam-distillable and fat-soluble, whereas the oviposition stimulus is water-soluble.

In nature, the dependency of the adults on such stimuli before oviposition occurs generally precludes the accidental oviposition on non-food plants. However, in the event that an egg is laid on a wrong plant, the larva issuing from the egg can either accept or reject the plant. For the larva to accept or reject a plant implies that a selective mechanism is present, and as in the adult, specific chemical stimuli mediate acceptance and continued feeding. The stimuli are called feeding stimulants or phago-stimulants. Rejection may occur when a plant lacks the specific feeding stimulant or when it contains feeding deterrents, as for example, in the plant Nicandra, which a hornworm larva soon abandons after feeding initially. The feeding deterrent from this plant has been isolated but unidentified as yet. The feeding stimulant has been tentatively identified as a glycoside.

# Some Aspects of Nutritional Studies on Grasshoppers

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Attempts have been made to formulate a diet which could be used to determine quantitatively the nutritional requirements of the migratory grasshopper (Melanoplus sanguinipes) and the two-striped grasshopper (Melanoplus bivittatus). However, none of the diets tried was successful without the addition of an aqueous lettuce extract. Studies were then initiated on the nature of the growth factor in the lettuce extract by adding various lettuce fractions to a chemically undefined diet and noting the effect on growth of newly-hatched grasshopper nymphs.

It was determined that the growth factor was water stable, stable to heat and oxidation, dialyzable, and adsorbed on charcoal. In addition, activity was still present in extracts passed through Dowex 1, an anion exchange resin, but not in extracts passed through Dowex 50, a cation exchange resin. However, a persistent loss of activity was noted in those fractions which were active. Efforts to concentrate the active factor by column chromatography with Dowex 50 and activated charcoal failed. Individual fractions from the charcoal column were relatively inactive, but some of the original activity could be recovered by recombination of the fractions.

It was decided that in this case, where difficulty was encountered in developing chemically defined diets, antimetabolites might be used to investigate certain phases of nutrition. Investigation of the inhibition of sterol utilization was suggested as an important area in grasshopper nutrition. Preliminary test with Melanoplus revealed that a need for sterol in the diet can be demonstrated with the chemically crude method that was described. Melanoplus can also use cholesteryl acetate and dihydrocholesterol, but cholesteryl chloride is utilized poorly. It is not known yet whether cholesteryl chloride will inhibit cholesterol utilization in these insects.

# Some Effects of Host Plant Nutrition on the Two-Spotted Spider Mite

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In studies at Beltsville, Maryland, to investigate the influence of host plant nutrition on the two-spotted spider mite, two effects were measured, reproductive rate and susceptibility to malathion.

Lima bean plants grown in quartz sand were used as the host plant. Nutrient solutions containing various levels of nitrogen and/or phosphorus and/or potassium plus constant amounts of all other mineral elements essential for efficient plant growth provided the various experimental levels of plant nutrition.

The reproductive rate of spider mites was found positively correlated to the nitrogen content and negatively correlated to the carbohydrate and phosphorus content of bean leaf tissue.

Mites produced more progeny on young leaf tissue than on old leaf tissue. This effect, within the same plant, was most pronounced when plants were supplied low levels of nitrogen as compared to plants supplied high levels of nitrogen.

Two-spotted spider mites were more susceptible to malathion when cultured on bean plants supplied high nitrogen levels and less susceptible to malathion when cultured on bean plants supplied high phosphorus levels.

Cross-sections of bean leaf tissue from plants supplied different nitrogen levels showed marked differences in cell size, arrangement and chloroplast content. Mite feeding in palisade and spongy parenchyma tissue was accompanied by obvious withdrawal of chloroplasts and other cell components.

# Development of a Chemically Defined Medium for the Axenic Cultivation of Screw-Worm Larvae

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For rearing larvae of the screw-worm (Cochliomyia hominivorax (Coquerel); Diptera, Calliphoridae) a synthetic medium was developed that contained casein, yeast extract, cholesterol, inorganic salts, water, and agar. This medium was further defined by replacement of casein with a mixture of L-isomer amino acids, and of yeast extract with a mixture of B-vitamins and RNA. Larval growth and development on the defined medium were nearly equal to that on media containing casein and yeast.

In further studies on individual requirements, larvae absolutely required thiamine, riboflavin, pantothenate, niacin, and choline for growth. Biotin and folic acid stimulated growth and were necessary for maturation to the adult stage. Pyridoxine and its analogs, pyridoxal and pyridoxamine, inositol, $B_{12}$, and carnitine had no effect on growth. Niacinamide spared niacin, but p-aminobenzoic acid had no effect on the folic acid requirement.

Larvae failed to grow in the absence of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophane, and valine. Cystine and glycine were required for normal growth. Ornithine or citrulline could not be substituted for arginine, homocystine or cysteine for methionine, nor hydroxy-proline for proline.

The larvae had a requirement for cholesterol that could not be spared by dihydrocholesterol, ergosterol, or B-sitosterol. Addition of corn, safflower, or cod liver oils to the diet inhibited growth, possibly by mechanical stresses. Choline was spared by lecithin, but not by betaine, ethanolamine, or carnitine.

Ribose, glucose, and maltose added to the diet inhibited growth, but glycogen and starch at 1/2% levels had a slight stimulatory effect. The inhibition from saccharides or low molecular weight appeared to result from osmotic stresses.

RNA was essential for larval growth, but could be replaced by a mixture of adenine, cytosine, and guanine. Uracil and uridylic acid inhibited growth.

# The Relationship of Lipids in the Diet with Special Reference to the Worker Honey Bee

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Data presented were obtained in the process of developing methods for studying lipid metabolism in the honey bee. The ultimate purpose of this research is twofold: (1) to obtain basic information on the physiology and nutrition of the honey bee during reproduction, growth, and development and (2) to find or develop a pollen substitute that will support adult bee nutrition and permit normal and sustained brood-rearing by the honey bee colony.

Feeding experiments were carried out with newly emerged worker bees 1 to 24 hours old. They were confined in small cages and fed the following four foods: (1) sucrose candy, (2) fat-extracted cottonwood pollen, (3) unextracted cottonwood pollen, (4) unextracted dandelion pollen. Bees were allowed to consume the test foods for seven days. Food was then removed from cages and bees held for an additional 24 hours on water only. Total and saponifiable lipids were determined in eight-day-old whole and eviscerated bees and compared to that of a whole newly emerged bee. Data in this summary report are the results of analyses done with eviscerated bees.

Bees fed the dandelion pollen diet had more than three times the amount of total lipids than did bees fed the other diets. The amount of total lipids in bees fed sucrose candy and the cottonwood pollen diets was no more than that present in a newly emerged honey bee.

Total saponifiable fat was three times more in bees fed dandelion pollen than in bees fed the other diets and twice that in a newly emerged bee. The amount of saponifiable fat in bees fed unextracted cottonwood pollen and sucrose candy diets was about the same. Saponifiable fat in bees fed fat-extracted cottonwood pollen was only one-half as much as that present in a newly emerged bee.

The saponifiable lipid content was about the same in bees that consumed the unextracted and fat-extracted cottonwood pollen diets; however, it was only one-third of the amount found in newly emerged bees.

Results of preliminary studies indicate that the adult worker honey bee is selective in its utilization of pollen lipids. Gas chromatographic analyses showed they use certain of the fatty acids and sterols in pollen while rejecting others.

At present we do not know enough about lipid metabolism in insects to interpret the significance of these data in the physiology and nutrition of the honey bee. Methods are now being developed at the Tucson Bee Culture Laboratory to investigate specific mechanisms involved in the transport, transformation and biosynthesis of lipids in the honey bee.

# The Role of Diet in Influencing the Storage and Fatty Acid Composition of Triglycerides During Diapause Development in the Boll Weevil

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Baton Rouge, Louisiana

The relative distribution of material in the major lipid classes of boll weevil body fat fractionated by silicic acid column chromatography is dependent on several factors including adult age, larval and adult diet composition, and diapause. The neutral glyceride and free fatty acid fractions constitute the bulk of body fat and account for as much as 90% of the extractable lipids of diapausing adults.

The tremendous increase in body fat of weevils preparing to enter diapause is due predominately to an increase in the triglyceride fraction. Newly emerged weevils have 2-6% body fat of which only about 2% is triglyceride. After 2-3 weeks of feeding, nondiapausing adults will have 6-10% body fat (40-60% of which is triglyceride), whereas diapausing adults have 18-25% body fat of which 75-85% is triglyceride. The triglyceride level drops during the winter when the insects are not feeding, and by the following June the fat content is down to 3% (containing 28-30% as triglyceride). Boll fed adults will accumulate more triglycerides than square fed, while adults feeding on both squares and bolls will accumulate an intermediate amount.

Gas chromatography of the fatty acids of the triglyceride fraction reveals that adult diet is the major factor controlling the type of fatty acid incorporated into the triglycerides. Square fed adults have approximately equal amounts of oleic and palmitic acids, the two major fatty acids of the boll weevil. However, boll fed adults have a much higher proportion of oleic. A secondary subtle influence of physiological state was also observed, because the amount of palmitic acid appears to be constant for any given diet and physiological state, while oleic acid is slightly higher in diapausing as opposed to nondiapausing weevils on a given diet.

# Nutrition and Vision in the House Fly

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House flies reared on CSMA media were compared with flies reared aseptically on carotene-free larval diet. Heads were extracted, chromatographed, and analyzed for vitamin A alcohol and aldehyde (retinene). Both vitamin $A_1$ alcohol and retinene$_1$ were found in sugar-fed adults reared on CSMA larval media, but neither was detected in flies from carotene-free diets. Movement of flies in monochromatic light, from 325 to 725 mμ, show positive but different responses to light. The repellency of 525 to 575 mμ light, found only if alfalfa is present, is not related to β-carotene content. Electroretinograms do give a larger response if carotene is present. Morphological differences in the compound eyes were not found.

Photographed by Boyd George

# The Function and Fate of Dietary Sterols in the House Fly

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Insects generally have been found to require a dietary source of sterol for normal larval growth and metamorphosis. Our work has pointed to two additional physiological roles for sterols in the house fly, Musca domestica L.: (1) A dietary source of sterol is essential for sustained viable egg production in the female fly; on a sterol deficient diet eggs are produced but hatch and viability are low. (2) Cholesterol is also involved in the mobilization and utilization of nutrient reserves associated with the initiation of ovarian maturation in the female fly.

The quantitative sterol requirements for the above physiological processes and the metabolic conversions that occur during growth, metamorphosis, and reproduction have been studied in this insect, using $C^{14}$-labeled sterols in conjunction with a variety of analytical tools, including reverse isotope dilution, gas-liquid chromatography, and spectroscopy, and employing aseptic rearing techniques and semi-defined larval and adult diets.

The utilization and metabolism of $C^{14}$-cholesterol has been studied using this compound as the sole source of sterol in the larval and adult diets. Subminimal quantities of cholesterol have also been used in the larval diet in combination with "sparing sterols" such as cholesterol, which will fulfill in part but not in entirety the sterol requirement of this insect. The utilization and fate of the "sparing sterol" has been investigated using $C^{14}$-cholestanol, and the metabolism of the minute quantity of essential cholesterol is currently under study, using high specific activity $C^{14}$-cholesterol.

Other species of insects, including the German cockroach (Blattella germanica), have been examined in relation to the patterns of utilization and the metabolic pathways for sterols found in the house fly.

# Boll Weevil Rearing

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Adult boll weevils are fed either small cotton bolls or an artificial diet utilizing cotton square powder, soybean protein, germinated cotton seed, sugar, salt, vitamins, ascorbic acid, and cholesterol in an agar base. The weevils lay eggs in the bolls or diet, and these eggs are then extracted by washing them through a graduated series of screens. The bolls have to be sliced or chopped up prior to the washing process.

After the eggs are cleaned to remove all trash, they are surface sterilized in 0.02% mercuric chloride. The sterilized eggs are then planted in a larval diet.

The larval diet consists of agar, water, cholesterol, salt, sugar, ascorbic acid, soybean protein, square powder, Brewer's yeast, amino acids, vitamins and mold inhibitors. After the larval diet is poured into petri dishes and allowed to harden, the surface is scratched by means of a sterile instrument. The sterilized eggs are placed in sterile water, picked up in groups of 70 eggs at a time, and placed on the scratched surface of the larval diet. Seventy 23-gauge hypodermic needles equally spaced over a 3-inch diameter chamber are used to pick up the eggs.

The larval diet and eggs are then incubated at 85° F. for 13 days, at which time the adult weevils begin to emerge.

The petri dishes containing adult weevils are placed inside a darkened box with glass jars fastened to one end. After the weevils crawl into the jars, they are removed and used for either testing or breeding stock.

# Boll Weevil Rearing

Louis A. Bariola, Entomologist
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College Station, Texas

Boll weevils are reared on laboratory media, consisting of cotton leaf meal powder, soybean protein, sucrose, agar, vitamins and other nutrients. Antimicrobial agents are added to reduce contamination. Adult weevils oviposit in small to medium size bolls. Eggs are removed by hand slicing the bolls into small pieces and washing it through a series of seives, catching the eggs on a No. 50 U. S. Standard Seive (opening is .0117 inches or 297 microns). Plant debri is removed from the eggs by a concentrated salt solution. The eggs are transferred to the media by an implanting machine, which uses vacuum to attach eggs to hypodermic needles, then air pressure to detach them and blow the eggs to the surface of the media. There are 78 needles on the implanter and approximately 50 to 60 eggs are put in each dish of media. The innoculated dishes have absorbent disks on them and the media is allowed to dry for 24 hours, then the solid top is replaced. When larval development and pupation have been completed, the tops are removed, the dishes are put in a small darkened box fitted with glass vials, and the weevils are collected from the vials daily. Newly emerged adults are kept in 1/2 gallon ice cream cartons, fitted with a screen top, and are fed cotton seedlings daily. Approximately 60 to 70 man-hours per week is spent on rearing the weevils. Approximately 2000 to 4000 weevils are reared weekly, with some weeks over 5000. Egg hatch is approximately 90%, and approximately 50% of the innoculated eggs produce adults.

# Boll Weevil Rearing

N. W. Earle, Entomologist
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Baton Rouge, Louisiana

Several types of containers may be used for rearing boll weevils. For small numbers of insects, vials individually capped with aluminum foil appear to be the best. Groups of 50 vials may be also covered as a unit with aluminum foil. Under these conditions, the yield of adults based on the number of eggs implanted can be expected to be around 80-85%. Disposable plastic petri dishes are more practical for rearing larger numbers of insects. When using petri dishes, only about 50% of the eggs reach the adult stage and microbial contamination is a more serious problem. This latter difficulty may be largely overcome by treating the eggs with 18% copper sulfate, which seems to be superior to the conventional egg sterilization solutions.

Cotton squares have been found to be satisfactory for routine feeding of the adult weevils. Under optimum conditions, from 12 to 13 eggs per female per day may be obtained from squares for a period of at least two weeks. Gravid females show a marked preference for cotton squares weighing 0.3 to 0.35 gm. (minus bracts). They will feed extensively on larger squares but lay very few eggs in them. There is evidence for the presence of an ovipositional stimulus in the squares that is distinct from the arrestant or attractant. Frozen squares have been found to be just as satisfactory as fresh squares for oviposition. There is evidence that weevils will actually oviposit more readily in frozen than in fresh squares.

Illumination of adults with light of short wavelengths tends to suppress diapause and enhance egg production. This effect is different from photoperiodism which controls diapause induction in this species. Photoperiod is most effective during the early larval instars. Although it is the adult insect that undergoes diapause, the adult stage is not responsive to photoperiod.

Diets developed for rearing larvae as well as for feeding adults contain an acetone powder of cotton squares as the main source of protein. Analysis of the amino acids and lipids in cotton plant parts have made it possible to develop diets that closely resemble the natural food of this insect.

# Boll Weevil Rearing

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The boll weevil rearing program at Florence is for the purpose of securing weevils for nutritional and other various studies and as such, is not a significant part of the research program. Consequently, the methods used have features of the methods from other laboratories with modifications required by local situations.

Larval and adult holding cabinets are modified walk-in refrigerators with the adults and larvae maintained in separate units. The diet preparation and egg seeding area is a part of a room used for other work.

The larval diet is a combination of those used at College Station and Baton Rouge. Copper sulfate is used to isolate the eggs from pellets composed of an adult diet which uses germinated cottonseed as reported by Dr. Vanderzant. Alternate feeding on cotton squares is essential for good egg production on this diet. The eggs are seeded in the larval diet in plastic petri dishes.

When adults begin to emerge, the open petri dishes are placed in a collection cabinet so that mass collection of the weevil is accomplished without additional labor.

The experience at Florence has shown two factors to be important in obtaining good yields. One is the avoidance of excess moisture in the larval dishes and the other is a uniform distribution of cholesterol in both the adult and larval diets.

Square powder, which is the basic ingredient in the diets, may be prepared by acetone extraction or air drying of ground squares. Dried egg white and egg yolk show promise as protein additions for use in both the adult and larval diets.

# Pink Bollworm Rearing

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The pink bollworm has been artificially reared on a wheat germ diet (Ouye, 1962, Jour. Econ. Ent. 55(6):854-7) since 1959. The prepared medium is poured into mustard dispensers and squirted into 2-dram vials (3 ml./vial) for individual rearing. For mass rearing, media are poured on trays and allowed to solidify. After a day or two, the medium is cubed into 1/4" pieces by forcing through 1/4" hardware cloth. Larvae are reared in 8-ounce drinking cups. These cups are infested in the following order: A layer of cotton packed on the bottom, a layer of cubed media, larvae, another layer of cotton, a layer of media, etc. Larvae used were transferred to cups with a small tuft of cotton. When the process is completed there are five layers of cotton interspersed with four layers of media.

The cups are then enclosed with a plastic lid and inverted onto trays; after 2 days the cups are turned right-side-up. After approximately 4 days, the cup lids are removed to allow for evaporation and placed in emergence cans which are fitted on the bottom with a circular-shaped hardware cloth about 2-1/2" high. Strips of paper-toweling serve as a pupation site at the bottom of the cage. The can lid is fitted with a cone-shaped screen. The apex of this screen is designed to hold a moth-collecting jar.

Oviposition cages are made from 1-quart paper food-containers having a diameter of 3-1/4". The top is fitted with screen. For feeding, absorbent cotton, semi-saturated with 5% sugar solution, is placed on the screened lid.

Individual rearing is nonaseptic, while mass rearing is semi-aseptic. In both types of rearing, antimicrobial agents are utilized. The most sensitive assay for detrimental effects of the above agents on larvae is rate of development. The amount of formaldehyde and methyl p hydroxybenzoate (M.P.) is 0.04% and 0.15%, respectively, while the concentration of measurable delay in larval development is 0.08% and 0.30%, respectively. In vial rearing, contamination is rare. When we do get contamination, it is localized in areas away from the media where condensation has taken place or in frass. Since pink bollworms metabolize the antimicrobial agents to noneffective metabolites, insects having larger frass would have a greater contamination problem.

# Rearing the Cotton Aphid

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College Station, Texas

The cotton aphid (Aphis gossypii Glover) has been cultured at the College Station, Texas, Laboratory since 1948. The culture is maintained on seedling cotton plants held in large-mouth bottles containing a complete nutrient solution.

The seedling plants are obtained from thick plantings of cotton seeds in tin trays of builders sand. Seedling plants 24 to 36 hours after emergence are preferable to older plants for food. The aphids are transferred to fresh food by pinching the infested cotyledons from their stems and placing them on the fresh food. The aphids are then allowed to transfer at will. Later the wilted cotyledons are removed. The culture should be placed on fresh food every four or five days. Overcrowded populations and bottles containing old honeydew covered plants produce the alate form.

The culture is reared in a refrigerator equipped with fluorescent lamps. The temperature is thermostatically controlled and held around 75° to 80° F. The evaporation of water from the bottles of nutrient solution containing the seedling plants has been sufficient to maintain the humidity.

New cultures may have to be started occasionally, the cause is chiefly due to mechanical failure of the refrigerator box causing overheating. Also, new cultures may have to be started as a result of insect diseases, or the presence of parasites, predators, and spider mites.

# Rearing Spider Mites

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Stoneville, Mississippi

As with insects, spider mites are most easily reared under natural or near natural conditions.

The greatest problem encountered in rearing spider mites is an adequate supply of host plants. Plantings of host plants should be made at intervals so as to have a supply available at all times.

Spider mites are reared either individually or en masse depending upon the use to which they are to be put. In life-history studies, leaf discs edged with "tanglefoot" floated in a tap water substrate is a simple technique. The use of clothes-pin-type cages is also very good. Cotton seedlings in vials of nutrient solution is also a good technique. If individual rearing is done, mites may be transferred by using a single bristle of a small camel's hair brush glued to the side of an insect pin extending about one-half inch beyond the point.

Mass rearing is done, usually in the greenhouse, where mites are transferred from infested to uninfested plants by placing an infested leaf on an uninfested plant allowing mites to transfer themselves, then allowed to develop naturally. Temperatures of 70° to 80° F. are adequate for most species.

The numbers of available rearing host plants for a food supply is usually the only limiting factor in the mass culture technique. Mass cultures have been made using lemons and oranges for a food supply by some workers using citrus mites.

# Technique for Rearing the Lygus Bug

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In the West, Lygus hesperus Knight is the predominant lygus species on cotton. Therefore, this species is reared at out laboratory.

The green bean rearing technique is used because it is simple, practical, and satisfactory for our purposes. Lygus are reared to provide test insects for the evaluation of insecticides, varietal resistance and measures of damage. Three or four man-hours per week are required to rear from one to two thousand adult insects. Although 34 successive generations have been reared entirely in the laboratory, we prefer to collect parent stock from the field and use the first laboratory-reared generation for our tests. This procedure provides insects of known age with no appreciable difference in tolerance or resistance from field populations.

There are two items which must be considered when obtaining green beans for the rearing procedure. They may be chemically contaminated or they may be of poor quality which results in early molding. However, we have experienced contamination only once in four years, and poor quality can usually be avoided by careful selection at the produce terminal.

The parent stock is collected by sweeping alfalfa with an insect net. The adults are collected from the sweep net with an aspirator and are then transferred to an oviposition cage containing beans. These cages are 8"x8"x12" wood frames covered with 30-mesh saran screen. Seventy-five bugs of each sex per cage give maximum oviposition.

After the green beans have been exposed to oviposition for 48 hours, they are transferred to one-gallon glass jars. Each jar is dated and set on the shelf in the rearing room for incubation which requires 6 days. A day or two before the eggs are due to hatch, fresh beans are added to provide food for the young nymphs. Nymphal development requires 15 to 18 days. Fresh beans are added every two days. The old feeding beans are removed at that time, but the beans containing eggs are left for 14 days. The nymphs are shaken from the old beans into the bottom of the jar when fresh beans are added. The pre-oviposition period for field-collected bugs varies from 9 to 29 days. The longer periods occur in the winter. Egg laying usually lasts about two weeks. A temperature of 78° F. with a relative humidity of 60% is maintained in the rearing room. We prefer to handle the bugs with an aspirator although $CO_2$ can be used satisfactorily if over exposure is avoided. We have never had trouble with disease in lygus rearing but use the following precautions: after use, the jars and cages are washed with soap and water; the jars are rinsed with acetone and the cages with water; and both are placed on metal racks outdoors where they are exposed to the sun until they are reused.

# Hessian Fly Rearing Technique

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West Lafayette, Indiana

At Lafayette, Indiana, greenhouse populations of four hessian fly races are being reared on susceptible wheat seedlings for research purposes and to evaluate wheats and barleys for resistance to hessian fly. Over 10,000 wheat and barley entries from six States are evaluated annually.

Adults emerging from infested wheat material, removed from the soil, are confined to susceptible wheat seedlings growing in flats covered with cheesecloth tents held up by 12-inch planting stakes. Eggs are generally laid during a one day period, after which the infested wheat innoculum and tents are removed and the wheat seedlings are allowed to grow at 65-70° F. under conditions of high humidity. The complete life cycle under greenhouse conditions takes approximately thirty days. Infested wheat material not ready for immediate use can be stored at 40° F. up to two years, and fifteen days after the innoculum is removed from cold storage the adults will emerge. With this method of rearing hessian fly, upwards to 3000 adults can be reared from one greenhouse flat of infested wheat.

Individual progeny studies are conducted in a similar manner using 4-inch pots instead of flats. Individual pairs of hessian fly are confined to pots containing wheat seedlings covered with small cheesecloth tents held up by four six-inch planting stakes. By seeding each pot with four wheat differentials the race phenotype of individual progenies can be determined.

# Rearing of the Wheat Stem Sawfly

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Bozeman, Montana

In brief, the life cycle of the wheat stem sawfly, Cephus cinctus Nort. (Hymenoptera: Cephidae), is as follows. The adults begin to emerge from the stubble in the latter part of June and remain active in cool weather until the latter part of July. They deposit eggs in the center of the wheat stem. These eggs hatch in about 10 days, and the larvae feed inside the stem until the latter part of August. At this time, as the plants mature and begin to dry out, the larvae chew a groove around the inside of the stem at or just above ground level. They then retreat below this girdle, plug the stem with frass, and spin a cocoon. They spend the winter as larvae and in the spring transform to a prepupa and pupal stage and emerge as adults from the stub.

In order to obtain adult sawflies for greenhouse work in the winter we dig wheat plant roots with the sawfly larvae in them and store them in cans. The plant roots are moistened and subjected to 10° C. for 90 days to break diapause and warmed to 70° F. to induce them to transform to adults.

More recently, interest in basic studies on wheat plant resistance to sawfly tunneling and cutting has stirred interest in rearing sawfly larvae on an artificial diet. At the Dominion of Canada Laboratory, Lethbridge, Alberta, Drs. R. Kasting and A. J. McGinnis (1962. Canadian Ent. 94(6):573-574) have been exploring this approach using different diets and rearing containers. They were unable to get larvae of less than 5 mgs. in weight to develop to maturity. They could get three-fourths of the larvae over 5 mgs. or more in weight to mature and spin a cocoon using a diet of casein, yeast extract, cellulose powder and other components. However, they were unable to get these larvae to transform to adults.

Because the larvae are apodous they are unable to move in a media mass but must be confined in a small tube. Kasting and McGinnis used 3/32" grooves in a plastic or wooden block covered with a plastic or wooden sheet and plugged at both ends with cotton. They found that the humidity in these rearing chambers must be high, so the wood blocks were soaked in water and the plastic blocks were held in a closed container above water. Even then, the larvae were moved to newly provisioned rearing chambers every 24 hours.

Artificial diets are not fully worked out, and some of the problems that still remain are as follows: A gap in the feeding period of the larvae is left because larvae of 5 mgs. or less fail to develop; high humidity requires transferral of larvae to new rearing chambers and creates a contamination problem; no larvae have been brought through to the adult stage. No larvae have matured on lyophylized wheat plants.

# Rearing Aphids Attacking Small Grains

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Stillwater, Oklahoma

It was necessary to rear greenbugs in large numbers in connection with studies on the development of greenbug resistant small grains. These aphids were used for manual infestation of small grain varieties in searching for resistant germ plasm and in subsequent tests to measure degrees of resistance of hybrids involving resistant parents.

Greenbug cultures were maintained on small grain plants growing in 6-inch pots. The greenbugs were confined to the plants by means of 5"x10" cylindrical cages closed at one end with muslin. The cages were made from clear cellulose nitrate plastic sheets (Pyralin), .020" in thickness, and polished on both sides. In making all of the cages for these studies the joints were sealed with acetone. Rectangular cages made of the same material which measured 10"x11-1/2"x21-1/2" also were used for rearing and for confining greenbugs on plants in test flats.

Average temperature of 75°F. was optimum for both greenbug and plant development. Light was supplied by fluorescent tubes for 16 hours, with 8 hours of darkness each day. Relative humidity varied from 50 to 60%.

These cages were used successfully for rearing greenbugs, English grain aphids, corn leaf aphids, and yellow sugarcane aphids.

In testing other materials as substitutes for cellulose nitrate plastic sheets, when this material became unavailable from the only known source, vinyl and cellulose acetate (Plastacele) plastic sheets were found to be toxic to both greenbugs and barley plants. Cellulose nitrate, 3 polyethylenes, ethyl cellulose, and Plexiglas plastics and saran screen were not toxic. In recent correspondence another source of cellulose nitrate sheets in desired specifications was found: Nixon Nitration Works, Nixon, New Jersey.

# Rearing the Alfalfa Weevil

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The alfalfa weevil overwinters in the adult and egg stages. Larval development and pupation occur in the spring. New adults leave alfalfa fields soon after emergence, spend the summer in a diapausing condition, and return to alfalfa fields in late fall for mating and egg laying. Host plants are essentially limited to a few legumes: alfalfa and sweet clover being preferred. Both larvae and adults utilize buds and leaves as food.

Both adults and eggs may be stored for months at 40° F., the adults in cardboard ice-cream cartons containing moist excelsior and a small bouquet of alfalfa, and eggs on moist filter paper or plaster of Paris. During 1962, we collected and stored approximately 24,000 adults and reared 6,000 primarily for insecticide screening and plant resistance work. The field collection of sexually mature adults in large numbers in late fall or early spring is not an easy or sure task.

The actual rearing now being done is on a very small scale and primarily for biological studies and some insecticide screening. This is accomplished simply by using fresh cut stems or bouquets of alfalfa in glass containers.

Efforts to replace fresh stems with a standard diet are promising. Extracts of fresh alfalfa prepared by Dr. Yamamoto, Insect Physiology Laboratory, Beltsville, contain a substance or substances that evoke a pronounced feeding response from adults. Fresh alfalfa killed in ether, powdered, and then reconstituted in agar is readily accepted by both first instar larvae and adults. Previous work with slurries and agar preparations of both alfalfa meal and fresh tissues had not been successful.

For continuous rearing, the normal presence of a 3 to 4 months diapause and one generation per year, presents a real problem. Last year we reared two groups through 4 complete generations and could have obtained 6. It was found that rearing first generation larvae under a short photoperiod of 8 hours light per day yielded non-diapausing adults which began laying eggs 30 days or less after emergence. Exposure to 12 hours or more yielded diapausing adults. This effect was not apparent in subsequent generations, but the average length of time from first adult emergence to first egg laying decreased with each succeeding generation.

# Rearing Potato Leafhoppers

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Potato leafhoppers are reared to test alfalfas for possible resistance to leafhoppers. The methods used to rear and transfer leafhoppers in testing alfalfas include:

Broadbeans, Vicia faba L. are grown in 4-inch pots in a greenhouse at 70° F. and are ready to use for food in about 5 weeks. Three plantings weekly assure a continuous supply of food. Leafhoppers are reared in cages in a second greenhouse at 70° F. To start cultures, several bean plants are first placed in each cage, then 10 to 15 adult leafhoppers swept from alfalfa fields are put in each cage twice weekly for 2 weeks. Thereafter, cultures are maintained by adding new bean plants and removing old ones from the culture cages as needed.

Only newly hatched nymphs are used in tests. They are obtained by putting 1 or 2 uninfested broadbean plants in each culture cage for 24 hours so that adult leafhoppers may lay eggs in them. On removal, the plants are held 9 days while the eggs incubate. Nymphs start hatching on the tenth day. A continuous supply of new nymphs is obtained by exposing uninfested bean plants daily. Small squares of paper having punched centers are used to transfer nymphs from broadbean to alfalfa without injury to the insects. With the aid of forceps, a square of paper (about 1/2 inch) is placed over each nymph, forcing it to walk onto the paper that is then quickly moved to the test plants. Leafhopper nymphs have been tested on both potted alfalfa plants and cut stem tips from alfalfa in the greenhouse and control chambers in exploring methods for locating possible resistance to the leafhopper. Nymphs are examined at the same hour daily for records of viability and development during tests. As nymphs transform to adults, they are removed and examined for sex.

# Grasshopper Rearing Methods

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Grasshoppers are needed for use in tests of insecticides, diets, resistant grasses, attractants and repellents, natural enemies, and for life history studies.

The Bozeman, Montana, Laboratory has developed a very good method of rearing grasshoppers. Eggs in moist sand in plastic cartons are held at about 38° F. The cartons maintain moisture in the sand and seem to inhibit mold, and are better than other methods tried. Eggs held over a year showed little decrease in viability. The eggs, still in moist sand, are incubated in a plaster-of-Paris hatching chamber at 84° or 90° F. The nymphs emerge through holes in the chamber into transparent collection tubes and are moved to a cellulose-acetate rearing tube 3"x18". Ends of the tube are closed with caps of 20-mesh screen. The grasshoppers are fed head lettuce and dry bran, and the rearing tube is put in a growth chamber held at 84° F. and 10 to 15% relative humidity. In the third instar the nymphs are transferred to a 24- x 24- x 18-inch rearing cage, 150 to 300 per cage. The sides and top, half of which is hinged, are made of 12-mesh screen in a wood frame. An 8-mesh screen bottom permits passage of droppings and bits of food. The rearing cages are held in a rearing room at about 90° F., and 25 to 35% relative humidity, and with daylight-type lighting. The 'hoppers stay in the rearing cages until they are needed. The cages, cage racks, and floor of the rearing room are cleaned each day. Once a week the cages are removed, and the entire rearing room washed with soap and water and disinfected with a 1 to 9 solution of Chlorox. Screen cages 12"x12"x12" are set over boxes containing sand and used for various experiments and for collecting eggs from laboratory-reared grasshoppers.

At Mesa, Ariz., grasshoppers are reared in a laboratory room at 75° to 80° F. and 25 to 35% relative humidity from November to early March and in a screened insectary without temperature or humidity control during the rest of the year. The basic cage for mass rearing is 16" square and 15" high, covered with 20-mesh screen, and with a painted board bottom. Two screen doors cover the front, the top one opening upward and the lower one downward. About 200-300 nymphs or 50-200 adults are used per cage. Flower pot saucers or metal pans are filled with moist sand and set on the bottom of the cage to receive eggs. Eggs are stored over a layer of moist sand in metal boxes 1" deep x 2-3/4" in diameter or 1-1/2" deep x 3" wide x4" long held at 42° to 45° F. They are incubated at about 85° F. in the same metal boxes. At hatching time the nymphs are allowed to emerge by transferring the egg containers to the floor of the rearing cage and removing the lids. The diet is succulent alfalfa, johnsongrass, and head lettuce, supplemented by filaree or hedge mustard when available. The food is changed and the floor of the cage cleaned daily. Small cylindrical glass tubes or screen cages are used to rear individual grass-hoppers. A satisfactory cage for individual oviposition and longevity records is a screen cylinder 3-1/2"x6" with one end closed and the other end set over moist sand in a small container.

# Laboratory Rearing of the Fall Armyworm at Tifton, Georgia, 1959-1963

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The fall armyworm, Laphygma frugiperda (J. E. Smith), was selected as a test insect because it is an important pest of forage crops. This insect has many host plants and in South Georgia, there is an abundant year-round source of natural foods; oats, rye, millet, corn, and grasses.

Oviposition: Eggs are obtained through the use of a one-gallon "cookie" jar oviposition cage. The jar is covered with a piece of clear plastic which contains several openings. Two openings are for feeding honey solution and water in cotton stoppered vials; two are ventilation holes stoppered with loose cotton. Two slits in the cover permit introduction of strips of paper on which moths deposit their eggs. The papers are removed daily. The eggs, on small sections of the paper, are held in Petri dishes for 48 hours. Black head-stage eggs are placed in the rearing cages.

Rearing cage: The rearing cage, made of 40-mesh wire with a supporting framework, measures 7 inches in diameter and 11 inches high. The bottom consists of an 8-inch clay flower port saucer containing about 1 inch of moist sand. A circle of 1/2 inch-mesh hardware cloth is fastened to the inside wall of the cage 1-1/2 inches from the lower end. The cloth holds the food above the sand and permits larvae to pass through to the sand for pupation. The cage has a tight-fitting metal lid. Eggs, 4 to 5 masses, are placed in the cage on a small amount of food. An increasing amount of fresh food is supplied daily for 13 days. On the 14th day most of the larvae move into the sand, but some pupate in the accumulated food. It has proven best to remove larvae and place them on sand at least 1 inch in depth in flat shallow trays, (9"x12"x1-1/2"). The trays are covered with aluminum screen wire held in place with spring clips.

Production of larvae and pupae: Thirty-six rearing cages, using the clay saucer produced 1,984 pupae, averaging 55 per cage. Trays were stocked with no more than 100 mature larvae. A total of 947 larvae transferred to trays from 5 cages, produced 509 pupae averaging 102 or almost double the number per cage using the clay saucer.

Discussion: Optimum conditions of light, heat, or moisture have not been determined. The rearing rooms are maintained at 80° F. and 70% relative humidity. High moisture conditions must be maintained to keep the food in good condition for 24 hours or longer, but moisture conditions must be low enough so that rot and excessive mold do not develop.

1/ Presented by K. J. Starks.

# Rearing of Southern Armyworms

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Southern armyworms have been reared at Brownsville, Texas, for 7 years. Battery jars lined with wax paper and with a filter paper covering the bottom are used as oviposition chambers. These jars contain five pairs of moths, cut lima bean plants, and 10% sugar water impregnated on cotton. The bean leaves and areas of the wax paper on which eggs have been deposited are removed from the battery jar and placed in a petri dish. The petri dish contains a wad of moist cotton to maintain a high humidity. The eggs hatch within 2 or 3 days. Upon hatching, the larvae are placed on lima bean plants growing in vermiculite. When the bean plants are consumed, the larvae are placed in large pans and fed lettuce and potatoes. The full grown larvae are allowed to burrow into moist vermiculite to pupate. The larval stage requires 15 - 16 days. Pupae are removed from pans of vermiculite, sexed, and placed in battery jars containing moist vermiculite and ramps to provide a resting place for the moths. The pupal stage requires 11 - 12 days. The adult moths are held in battery jars containing ramps and 10% sugar water impregnated on cotton. The LT/50 for adults is about 1 week. Southern armyworms have also been reared on a synthetic media designed for bollworms and another designed for cabbage loopers. Larvae grow much faster on these artificial media if the newly hatched larvae are first allowed to feed on living bean plants for 2 days.

# A Mass Rearing Diet for the Sugarcane Borer

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Requirements of a satisfactory diet are: (1) The diet must be well-rounded and nutritious so as to insure high survivorship in all life stages, at least normal fertility and fecundity, and a developmental period not excessively long. (2) The diet must remain gustatorally acceptable with a minimum of contamination by microorganisms throughout the normal larval life span of 25 to 35 days so as to avoid the need for transferring larvae to fresh medium. (3) The diet should be easy to prepare on a large scale. (4) The diet should be low in cost.

Diets devised for the sugarcane borer were generally intended for small-scale laboratory studies. In each case preparation was complex and a large number of dietary constituents were utilized. A need for simplification of the diet was evident.

Our approach to the problem was based on the premise that corn top powder, dried at a moderate temperature and then milled, would in itself furnish adequate nutrition for sugarcane borer larvae.

The dried powder is added to an agar-water carrier in a proportion approximately the solids normally found in corn or sugarcane plant tissue. The medium is buffered at a pH of 5.8, a compromise between the pH of the leaves eaten by first and second instar larvae and the stalks eaten by older larvae. Finally, a formalin-tegosept antibiotic system is now being used to control bacteria and fungi.

It may be seen that the new diet combines low cost and ease of preparation with proper nutrition.

We are presently evaluating the need for additional sucrose and ascorbic acid in the borer diet. The basic experimental diets are compared with a modification of Panand Long's diet in which whole corn top powder is employed rather than acetone extracted top tissue.

# A Method for Laboratory Handling of Several Corn Rootworm Species

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There is no published rearing method for the two species of rootworms which are serious pests in the corn growing areas, Diabrotica virgifera (LeConte) and Diabrotica longicornis (Say). In order that work may continue through the winter, the following methods were developed. They are not considered perfected but are a first successful attempt to raise these insects through their life cycle.

The adult rootworms are field collected from infested fields using a vacuum collector. This specially designed collector is a Singer portable vacuum cleaner with a pint carton cage enclosed in the wand. This is carried into the field by utilizing a portable 110 V. generator and extension cords.

The beetles are confined in a 1 cubic foot screen cage. They will feed readily on lettuce, summer and winter squash fruits, green ears of corn and immature kernels. Eggs are collected on moist Cellu-Cotton placed below the screen at the bottom of the cage. Egg papers are stored in polyethylene bags at approximately 40° F. Incubation of cage papers in moisture tight containers at 86° F. will yield larvae in 2 to 3 weeks. Hatching proceeds continuously for 60 to 70 days.

The larvae are reared on corn germinated on agar plates using a modification of the methods of Dr. J. H. Bigger and Dr. R. B. March (1943. Jour. Econ. Ent. 36(2):349) for the southern corn rootworm. Two to three hybrid corn seed, treated with Thiram or sterilized in 10% Clorox, are germinated at 86° F. on a sterile 1-1/2% agar plate. Six first instar larvae are placed on the newly germinated seed and incubated at 70° F. The larvae are transferred about three times during the 40-day larval stadium. Prepupae dig out pupation cells in the agar. They are left there to pupate or are moved to moist Cellu-Cotton lined boxes. The pupal stadium lasts about 17 days. When adults emerge, they are placed in 2"x9" cellulose butyrate cages. These cages have been used to collect eggs from small numbers of beetles of all three species of rootworms and the striped cucumber beetle.

# Rearing the European Corn Borer in the Laboratory

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The following procedure has been used successfully in rearing the European corn borer. Pupae are placed for emergence in a screen wire cage which has a 1/4" mesh hardware cloth top. Waxed paper sheets are placed over the hardware cloth. High humidity and contact moisture appear to be necessary for adequate emergence. The cage is placed in an incubator maintained at a temperature of 80° F. and 70-80% r.h. for oviposition. (Mating is improved if the cage is maintained in a bio-climatic chamber with conditions simulating a normal June day.) Moths oviposit on the waxed paper sheets which are removed daily. Disks of waxed paper containing the egg masses are placed in small screw-capped jars which are placed in the incubator for hatching. Newly hatched larvae are transferred into individual vials containing laboratory diet, which are then placed in an incubator set to operate at 80° F. and 70-80% r.h. Pupation begins in 13-14 days and first emergence of the new generation begins about the 23d day. In order to prevent diapause, lights are left on continuously in the incubator.

The laboratory diet contains leaf factor prepared by cutting the whorl and leaf of the susceptible inbred corn, WF9, and drying it at 70° C. under a vacuum of 15 inches of mercury. It is then powdered in a Wiley Mill and stored in sealed plastic bags which are kept in a refrigerator. The diet formula is given below.

| | | | |
|---|---|---|---|
| Distilled water | 285 ml | Wheat germ oil | 0.5 ml |
| Agar | 6.6 gm | Salt Mixture No. 2[2/] | 1.3 gm |
| Glucose | 10.5 gm | Choline chloride | 0.12 gm |
| Casein | 10.5 gm | Brewer's yeast | 6.9 gm |
| Cholesterol | 0.9 gm | Leaf factor | 13.8 gm |
| Corn oil and Vit. "E"[1/] | 0.5 ml | Sorbic acid, stock solution[3/] | 3.0 ml |

This quantity is adequate to feed 75-100 larvae to maturity at a cost of 2-1/3¢ per larva for labor and materials.

The fecundity of early emerging moths from large rearings was at about the same level as moths of field origin. Later emerging moths were weaker and short-lived.

Field and laboratory tests show that larvae hatched from egg masses laid by moths reared in the laboratory one generation establish and grow as well as progeny from field sources.

1/ Mazola oil containing 1% alpha tocopherol
2/ Nutritional Biochemicals, Cleveland, Ohio
3/ Stock solution -- 23 gm sorbic acid into 75 ml 95% ethanol

# Rearing Requirements for Mosquitoes

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Three species of mosquitoes are colonized at Orlando - the common malaria mosquito (Anopheles quadrimaculatus), the yellow fever mosquito (Aedes aegypti), and the salt marsh mosquito (Aedes taeniorhynchus). Except for special behavioral or physiological studies, rearing techniques are similar for these species. Adults are held in screened rectangular cages varying in volume from 3 to 12 cubic feet and illuminated by natural daylight coming through windows and fluorescent lights operated during working hours. Adults are fed on 20% honey solution and rabbits. Eggs of quadrimaculatus, aegypti, and taeniorhynchus are deposited on the water, on moist filter paper, and on moist sphagnum moss, respectively. Embryonation periods of 4 to 7 days are required for aegypti and taeniorhynchus eggs, whereas quadrimaculatus eggs hatch in 2 days. Approximately 250 Aedes and 500 Anopheles larvae are reared per 10-inch porcelain pudding pan containing spring water for quadrimaculatus, tap water for aegypti, and a mixture of 2 parts river water and 1 part sea water for taeniorhynchus. Beginning one day after hatching, larvae are fed a mixture consisting of 10 parts ground dog biscuits and 1 to 2.5 parts Brewer's yeast. A. aegypti and taeniorhynchus receive this mixture as a slurry poured directly into the rearing pan. A. quadrimaculatus larvae are fed twice daily by sprinkling the dry mixture on the surface of the water, since excess food causes a scum, and too little food results in starvation, uneven development, and cannibalism. Aedes larvae pupate in 6 to 8 days; quadrimaculatus in 10 to 15 days. Pupae are separated from the larvae manually or automatically and placed in adult cages for emergence.

The Red Rose Dog and Puppy Food® utilized as larval food contains a minimum of 23% crude protein, 7% crude fat, and 4.5% crude fiber. The ingredients are as follows: Meat meal; animal fat (preserved with butylated hydroxyanisole); toasted soybean meal granules; corn flakes; wheat flakes; Zwieback; dried tomato pomace; dried beet pulp; dried buttermilk; dried whey solubles; Brewer's yeast; dehydrated cheese; thiamine; riboflavin; niacin; vitamin E supplement; vitamin A palmitate; vitamin $D_2$ supplement; trace amounts of manganese oxide, iron carbonate, copper hydroxide, cobalt carbonate, potassium iodide, and defluorinated phosphate.

The results of recent field investigations indicate that the colonization of quadrimaculatus has brought about important behavioral changes in the male which prevent it from competing successfully with wild males for the insemination of females in a wild population.

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# Rearing and Maintenance of a *Culex tarsalis* Coq. Colony

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Three strains of the mosquito, *Culex tarsalis*, are maintained at the Corvallis, Oregon, Laboratory--one insecticide-susceptible strain and two resistant strains, DDT and malathion. Maintenance is briefly described below:

A. Adults
   1. Food: Males - 10% sugar-water or honey. Females - Fed on mice, chickens or other warm-blooded hosts. Also, honey and nitrated blood (1-9) warmed to 33-35° C. Works fairly well.
   2. Rearing cages: Size not critical but 16"x16"x16" screened with 14-18 mesh wire with cloth sleeve very convenient. "Standard house fly cages" satisfactory also.
   3. Light: Have been reared with no light control but present methods use 14 hours. Controlled lighting apparently necessary in some strains.
   4. Temperature and relative humidity: 75° F. and 60% RH works very satisfactorily, but can be varied--minus five to plus ten.

B. Eggs
   1. Females will lay on any type water but perfers tap to distilled.
   2. Incubation period at 75-80° F. approximately 48 hours.

C. Larvae
   1. Rearing temperature is not critical but 75-85° F. Very good.
   2. Container size: Not critical - larger ones work better than smaller ones. Pan with diameter of 14.5 inches with 2-3/4" water depth will carry 2000 larvae.
   3. Food: Nutritional requirements not known but foods with high protein content work well. Examples:
      a. "Hi-Protein supplement pellets". Powdered. (Nesco Feeds, Mont.)
      b. Powdered liver - raw or defatted substance.
      c. Powdered soybean, split pea, and commercial enriched yeast, equal parts of mixtures of yeast plus a or b. (Dog pellets not recommended). Amount of food depends on temperature, number of larvae, etc.
   4. Aeration: Not necessary but improves rearing under many conditions.
   5. Developmental period: Depends on food and temperature, but 7 to 10 days at 26° C. with foods under point 3 above.

D. Pupae
   1. Development at 26° C.: 36-48 hours.
   2. Separation from larvae: Eye dropper, wire strainer or "mechanical separator" for large-scale effort.

# The Effect of Different Levels of Illumination on the Life Cycle of the Face Fly

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Colonies of face flies are being maintained under three different levels of illumination, namely 5-, 50-, and 500-foot candles. General Electric daylight fluorescent lamps are used as the source of illumination. The photoperiod consists of 16 hours of light and 8 hours of darkness. After two generations the results indicate that there was no difference in the development time of the larvae or pupae or in the percent of adults that emerged, the sex ratio or the percent of abnormal adults. The colonies will be maintained under these conditions for six generations, after which the adults will be used in tests to determine the attractiveness of radiant energy in the ultraviolet and visible part of the electromagnetic spectrum.

# The Use of Light in Rearing Anopheles quadrimaculatus Say for Behavioral Studies

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Field tests have been conducted with Anopheles quadrimaculatus Say at Orlando, Florida, to evaluate the effect of releasing sterilized males in a wild population. After several releases appeared negative, it was believed that there was possibly a behavioral difference between the mosquitoes reared in the laboratory and those present in the field. In order to study some of the mosquito's crepuscular activities, particularly mating, during the regular working hours two illumination boxes (24"x18"x30" high) were constructed in which the level of illumination could be regulated by General Electric daylight fluorescent lamps attached to the ceilings of the boxes. One of the boxes (low intensity) was equipped with lamps so that the level of illumination was either zero or ten foot candles. In the other box (high intensity) there were five levels of illumination, the maximum level being 120 foot candles. In both boxes sunset occurred at approximately 1:30 p.m. and sunrise 2:30 a.m. Two ice cream cartons, painted black on the inside and provided with a 1"x1-1/2" hole in each end, were placed in each cage for the mosquitoes to hid in while the lamps were in operation. The tests indicated that (1) the crepuscular activities of the Orlando laboratory strain can be shifted to any desired time of the day by use of an artificial illumination system as in the high intensity box; (2) mating can be observed with the laboratory strain; (3) the percent of laboratory females inseminated, taking blood, and ovipositing may possibly be increased by using an artificial illumination system as in the high intensity box; (4) the use of the ice cream cartons as a resting place during the light period does not appear to affect the response of the laboratory strain; and (5) the environmental conditions used in the high intensity box are apparently not conductive to mating for the wild strain.

# Rearing of Face Flies

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The face fly, Musca autumnalis DeGeer, a serious pest of livestock has been successfully reared in the laboratory since the summer of 1960. This insect was difficult to rear at first since nothing was known of its nutritional requirements. When the adults were fed a milk and sugar diet they would not oviposit and did not live very long, whereas a more varied diet resulted in oviposition and greatly extended longevity. Also the face flies would not readily oviposit except in the presence of sunlight or high artificial illumination. Cow manure, the natural larval medium, was the only material found that readily stimulated oviposition and sustained larvae to maturity.

A culture can be started by netting adults in the field about cattle or by collecting larvae from the manure. The adults are held in a rearing room under continuous illumination from two 275-watt Westinghouse RS sun lamps and two 400-watt G. E. F96 PG17CW fluorescent lamps. The flies are confined in ordinary house fly rearing cages, 10"x10"x10" or larger and the room is maintained at a temperature of 80° F. and relative humidity of 55%. The diet given to the adults contains a skimmed-milk and sugar solution at the ratio of 2:1 V/V, about 0.35 gram of a "Diamalt" solution containing brain-heart infusion, and pollen. This solution is made periodically as follows: To 50 ml. of "Diamalt" is added 2.5 ml. of a 7.5% W/V water solution of brain-heart infusion, 2.5 ml. of a filtrate of a water solution containing 37 grams of pollen in a total volume of pollen and water to equal 200 ml. This solution is filtered through two layers of cheesecloth. There is always present a small cup of citrate beef blood which now replaces raw ground beef originally used. Also fresh cow manure, which is a needed food as well as an oviposition medium, is furnished daily.

Adult female face flies 6 or more days of age oviposit in the cow manure, the eggs being laid singly in upright position just below the surface of the manure. The dishes of manure with eggs and young larvae are removed daily. More fresh manure is usually added since the larvae grow rapidly. On the 4th day they migrate from the manure to a thin layer of sand in which they pupate. The white-colored pupae are collected and adult emergence occurs on the 8th day after pupation. A culture well acclimated to laboratory conditions can produce 5,000 to 10,000 face flies per day. Progress is slow on the development of an artificial larval diet for use in place of manure. However, with the knowledge gained to date on laboratory rearing, it is believed that mass-rearing could be accomplished now if the necessary space and equipment were available.

# Rearing Methods of House Flies

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House flies can be reared on a variety of media, but strains reared on different media may vary greatly in susceptibility to insecticides. As a result in the early 1930's the National Association of Insecticide and Disinfectant Manufacturers (now called the Chemical Specialties Manufacturers Association) developed standardized rearing methods which called for a larval medium (called NAIDM or CSMA medium) produced by the Ralston Purina Company. Currently, this medium contains 26.67% of alfalfa meal, 40% of Brewer's dried grain, and 33.33% of soft wheat bran. Twelve ounces of this are added to 750 ml. of an aqueous suspension of yeast and malt in water in a glass battery jar 6" in diameter and 9" high. About 2000 eggs are set in each jar. Larval development is usually completed in 6 to 7 days. The larvae pupate at the top of the medium and this portion is removed and air dried. The pupae are separated from the chaff, and placed in shallow dishes which are placed in a cage large enough to provide at least 1 cu. in. of space per fly. Adult flies are furnished food containing 5% spray-dried non-fat milk solids and 2% granulated sugar thoroughly dispersed in water. Eggs for the next generation are collected on food or other oviposition medium from flies not more than 8 days old.

A modification of the standard method is used at Orlando. Our house flies are reared in are reared in a room at 78-82° F. We mix 24 qts. of the CSMA dry medium with 8 qts. of oat hulls and 18 qts. of water in a tub; 4-qt. portions are transferred to 2-gal. jars lined with plastic bags. Yeast and malt are omitted. About 2000 eggs are placed on the medium and the surface lightly sprinkled with about a half-pint of water to reduce the temperature. If the jars later become uncomfortably warm to the touch, another half-pint of water is added. After pupation is complete, the top of the medium is transferred to a pan of water, where the medium sinks to the bottom and the pupae float. The pupae are strained off, washed, allowed to dry, and placed in colony cages in pint paper cups. Plastic bags containing the old medium are discarded. The jars are sometimes reused without washing. Eggs are obtained by placing a cup of old medium in a cage of breeding flies.

Instead of the liquid adult food recommended in the CSMA method, a dry mix containing 6 parts of granulated sugar, 6 parts of powdered non-fat dry milk, and 1 part of powdered egg is placed in paper cups in the adult cage. Water is provided in Mason jars fitted with paper towel wicks. The dry mix does not spoil, so enough can be supplied at one time to last 2 or 3 days, thus eliminating the need for week-end feeding. House flies are one of the easiest of insects to rear. This and their short life cycle accounts for their extensive use as test insects.

# Laboratory Colonization of the Horn Fly

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The successful laboratory colonization of the horn fly (Haematobia irritans (L.)) depends primarily on two factors: (1) Adult diet and (2) adult environment. The development of the adult diet has been described by R. L. Harris (Nature 196(4850):191-2). The diet that gives the most consistent results is a mixture of 1 part bovine blood and 1 part beef juice, plus 1 mg. streptomycin, 1000 units of penicillin, and 250 units of mycostatin per ml. of diet. The blood used is drawn from the vein of a steer into a transfusion bottle containing 120 ml. of ACD anticoagulant solution, to which 480 ml. of blood is added. Blood collected at the slaughter house and mixed with ACD solution has been tested, but is inferior to the veinous blood. The beef juice is obtained by centrifuging fresh ground beef muscle. The beef muscle yields more juice if it has been frozen.

Each of the cages used for colony rearing is made from a 4-inch length of a plastic cylinder 6" in diameter. The top and bottom of the cage consist of plastic sceen, and legs 1/2" long facilitate air circulation in the cage. A piece of lightweight unbleached muslin is placed on top of the cage, and the flies are fed by placing on the cloth a cotton pad soaked in the diet mixture. A plastic dish placed over the pad retards evaporation. The pads are usually replaced twice a day.

Temperature and relative humidity are important factors in the successful rearing of horn flies. A temperature of 90° F. has given the most satisfactory results. The adults live longer at 80° F., but produce about one-fourth as many eggs. Flies used for reproduction are held at 90° F., and those to be used in other tests are held at 80° F. Relative humidity is probably the hardest factor to control, because of the influence of the moist diets. If the relative humidity is too high, the flies become entangled in their droppings and die. However, if it is too low, the diet pads become dry and the flies die of dehydration. The best results have been obtained when the relative humidity is maintained between 60 and 80%. The influence of light has not been investigated, but undoubtedly is important. The flies are held under continuous fluorescent light.

Eggs are laid on the muslin and the plastic screen. They are collected twice daily and placed in cow dung. The dung is collected fresh, and is frozen to avoid insect contamination and for storage, but is never stored more than 1 week before use. Seven days after the eggs are planted, the pupae are removed from the manure and placed over moist cotton.

# Colonization of the Little House Fly, *Fannia canicularis*

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Corvallis, Oregon

A colony of the little house fly has been maintained at the Corvallis Laboratory for approximately one year. Present known methods are not totally adequate. The methods briefly described below have allowed a gradual increase in numbers.

A. Adults
   1. Food: 50% honey-water applied to the outside of the screen three times per week. Dry food (petri dish) powdered milk and sugar, 50/50 ratio. Free water supply.
   2. Rearing cages: Size not too important but recommend a cage at least 18x18x18 inches for stock colony. Smaller cage adequate for experimental work.
   3. Light: Present colony exposed to 16 hr. daylengths. Little known about effect of light.
   4. Temperature: 75-80° F. satisfactory for adults.
   5. Relative Humidity: 50-60% may be adequate.

B. Eggs
   1. Flies lay eggs on animal feces or decaying organic matter. Presently egged on 3-day old alfalfa meal-water mixture or the extract therefrom.

C. Larvae
   1. Container not critical. Gallon glass jar used. CSMA medium fairly satisfactory if moisture content is controlled and heating avoided. Larvae will develop at 70° F. but 80° F. recommended.

D. Pupae
   1. Larvae need a drier environment for pupation. Wood shavings have been used with good success.

E. Rearing rooms.
   Separate but adjoining rooms for larval and adult rearing suggested.

F. Nutritional requirements not known for either larvae or adults.

# Rearing of Cockroaches

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Beltsville, Maryland

Field-collected cockroaches were maintained at Beltsville on dry alfalfa and bran mixture and water starting in 1938. Periplaneta americana (L.) and Blattella germanica (L.) were colonized in 1940. At present P. americana is reared in screen-covered 28-gage galvanized-iron boxes 14"x18"x7". Larger drums can also be used. Small cultures can be reared in large glass containers. Inverted pint-size cartons with V-shaped openings in the open end are provided to give additional resting surfaces. Coiled or bent pieces of hardware cloth can also be used. The insects are confined by petrolatum or mineral oil on inside vertical surfaces. Water is provided in vials plugged with cotton or by means of beakers inverted on cotton. The food is discussed below. Egg capsules deposited by adult females are collected weekly and set aside to start new cultures on hatching 4 to 6 weeks later. Nymphs mature in 4 to 6 months at 75° F. and 60% relative humidity.

B. germanica is usually reared in glass battery jars although metal containers can also be used. Adult females are segregated in wire cages placed in a battery jar and the small nymphs that hatch migrate to the jar thus starting new cultures. Nymphs mature in 6 weeks and rear well at 80° F. and 70% R. H.

Additional species under culture by similar methods are:

| | |
|---|---|
| Supella supellectilium (Serv.)* | Nauphoeta cinerea (Oliv.) |
| Blattella vaga Hebard* | Eurycotis floridana (Walk.) |
| Blatta orientalis L.* | Blaberus craniifer Burm. |
| Leucophaea maderae (F.) | Blaberus giganteus (L.) |
| Periplaneta australasiae (F.)* | Blaberus discoidalis (Serv.) |
| Periplaneta brunnea Burm.* | Diploptera punctata (Esch.) |
| Periplaneta fuliginosa (Serv.)* | Byrsotria fumigata (Guerin) |

*deposit egg capsules, all others are viviparous.

The wood roaches, Parcoblatta pennsylvanica (DeG.) and P. uhleriana (Sauss.) have also been reared and P. fulvescens (Sauss. & Zehnt.), P. lata (Brunner), P. virginica (Brunner), as well as Ischnoptera deropeltiformis (Brunner), Panchlora nivea (L.), and Pycnocelus surinamensis (L.) have been maintained.

Cockroaches are presently fed a 1:1 mixture of Purina "Dog Chow" and "Lab. Chow", the combined ingredients are: crude protein, fat, and fiber, ash, meat-bone meal, wheat germ, fish meal, alfalfa, cane molasses, liver meal, beet pulp, corn grits, ground oat groats, ground yellow corn, oat middlings, ground wheat, soybean oil and meal, cereal food crumbs, whole whey, animal fat, vitamin $B_{12}$, E, and riboflavin supplements, vitamin A oil, thiamin, niacin, folic acid, deactivated plant sterol, Brewers' yeast, calcium pantothenate, choline chloride, defluorinated phosphate, iodized salt, ferric ammonium citrate, manganese sulphate, and traces of manganous, iron, and copper oxide, and cobalt carbonate.

# Colonization of the Stable Fly

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Lincoln, Nebraska

The stable fly can be colonized without difficulty. The methods used at the Lincoln, Nebraska, Sublaboratory of the Kerrville, Texas, Laboratory are described.

The temperature and relative humidity in the rearing room are 80-85° F. and 50-60%, respectively. Flourescent lights are on continuously. The adults are held in cylindrical screen cages 14" in diameter and 22" long. This size cage will accommodate 2000. Adults are fed citrated beef blood. Cotton pads saturated with blood are placed on top of the cages once daily. Eggs are collected in a dish of medium placed inside the cage or a dish containing a pad of cotton covered with a black cloth and saturated with a 2-5% ammonia solution. The dish is placed beneath and the pad is in contact with the screen of the cage.

Larvae are reared in a mixture of CSMA Fly Larval Medium and fluffy, soft-wood shavings (4:1 ratio by weight). The mixture is moistened to the point that water can be squeezed from it, but water should not collect in the bottom of the container. A variety of containers can be used. The maximum depth of the medium should be about 4 inches. It is stirred daily and water and CSMA are added when or if needed. Pupae are floated from the medium, dried, cleaned, and held in the rearing room. The developmental periods are as follows: incubation 1 to 1-1/2 days; larval 8-11 days; pupal 5-7 days; preoviposition approximately 7 days.

Some difficulties frequently encountered in maintaining a colony are: (1) lack of sufficient humidity in the room, (2) starvation and overcrowding of adults, (3) failure to place eggs in larval medium before hatching occurs, (4) too much or too little moisture and food in larval medium, (5) overcrowding of larvae, and (6) dessication of pupae after they are separated from the larval medium.

# Nutrition and Rearing of the Screw-worm

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Artificial culture of the screw-worm was first reported by Melvin and Bushland in 1935. The basic formula of 2 parts lean-ground meat, 2 parts water, 1 part citrated blood and 0.24% formalin has been used very successfully to the present day with minor modifications. Following the successful field trial of the sterile-male technique on Curacao in 1954, research was directed to economical mass-production of high quality insects. Various plant protein sources tested, such as soybean meal, peas, and other beans were unsatisfactory in the larval diet as were animal protein sources such as tankage, dried blood, and fish meal. However, fresh trash fish and frozen whale meat showed promise. Whale meat, though not as satisfactory as horse meat, was used in the finishing diet for the eradication program in the Southeast because of the cost advantage of 13¢ per pound compared to 25¢ for horse meat. A starting medium (fed the first 24 hours) in which plasma replaced the blood improved larval size and growth.

Major changes in rearing involved equipment used. Wash tubs were first replaced with 18"x26"x1" trays to conserve space, followed later by 4'x5' vats with built-in heating devices. Small colony cages holding 500 flies were replaced with 6'x6'x3-1/2' cages holding 56,000 flies. Egg production formerly obtained by containing 10 to 15 flies in vials with meat was simplified by the development of an ovipositional stimulant mixed with meat and placed on a temperature controlled vat. Other details of the mass-production techniques are illustrated in the literature available and also in the movie "Round-up".

During the Southeast Eradication Program almost 25% of the production was lost due to pupal mortality. Recent studies show this loss undoubtedly was due to failure to protect the highly susceptible full-grown larval and the later prepupal stages from desiccation. Correction of this situation in the current program has reduced losses to 5% or less. Development of a 50% fish flour substitution offers potential savings of 20 to 40% of the larval diet costs without sacrificing quality of the larvae produced. Labor plus meat costs in the current program are averaging $217 per million compared to $700 for the Southeast Program due to improvement in plant design, cheaper medium, improved proficiency of plant personnel, and production at a level approaching optimum utilization of the facility.

# Rearing Cattle Grubs on an Artificial Medium

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Attempts to colonize either of the two important species of cattle grubs, Hypoderma lineatum and Hypoderma bovis, have met with little success. The adults take no food and live only a short period of time. Fertile eggs can be obtained but only after considerable effort. The larvae spend their entire life in the host animal and remain in this form for nearly one year. Rearing efforts described below involved only the young larvae of Hypoderma lineatum obtained from cattle.

1. Larvae excised from the gullet and placed in a physiological saline, penicillin, streptomycin, and mycostatin "wash" and allowed to remain therein for 1-1/2 - 2 hours.

2. L-Y rearing medium

   A. Lactalbumin hydrolysate solution (plus numerous minerals, salts, etc.)
   B. Yeast extract solution
   C. Hank's balanced salt solution
   D. Beef serum
   E. Antibiotics penicillin, streptomycin, mycostatin

3. Sterile petri dishes which may or may not be slightly tilted

4. Incubate at 38° C., observe daily under the scope

5. Transfer every 3rd or 4th day

By the above procedures larvae may survive as long as one month.

Although admittedly these data are meager they do indicate that the larvae might possibly be reared under laboratory conditions.

Many problems have been encountered, such as contamination, physical and chemical changes in the medium etc., and most of these have been only partially overcome. The basic problem which still confronts the researcher, however, is that of maintaining the larvae over a sufficient period of time to allow for the determining of nutritional requirements.

# Colonization of *Culicoides*

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Denver, Colorado

The present colony has been maintained for about 3-1/2 years without the addition of wild material from the field. About 9 months ago the colony was moved from Kerrville, Texas, to Denver, Colorado, and as yet has not become fully adapted to its new environment.

The duration of the life cycle in the laboratory is now about 27 days; pre-blood meal - 1 day, pre-oviposition - 3, egg - 2, larva - 18, and pupa - 3. The required blood meal for the female is provided by applying a colony cage to the sheared belly of a restrained (upright) rabbit. Water and sugar are provided, the sugar by means of a cotton wick and a 5% honey solution.

Laboratory conditions for larval development simulate the normal breeding area in the field. The larvae are semi-aquatic. A larval pan is set up with water-soaked vermiculite, 250 ml. of cow manure, and 10 ml. of soil. Sufficient vermiculite is used so that channels can be formed with the intervening islands protruding slightly above the 1 inch water depth. The initial 250 ml. and the subsequent daily increments of 50 ml. of cow manure are mixed in a blender before use. A mechanical paddle for each larval pan prevents the formation of surface scum. Fifteen larval pans each 25 x 30 inches are held on a three-shelved rack. Since *Culicoides* pupae float in water, on pupal formation a pan is flooded for two hours and then overflooded through a notch at the rear of the pan into a movable trough that leads to a 100 mesh sieve. The few larvae that are flooded over are separated from the pupae by means of a 35 mesh sieve.

Frozen cow manure several years old can be utilized. Eggs can be held satisfactorily for over one month when refrigerated at 50° F.

# Laboratory Colonization of the Tropical Horse Tick *Dermacentor nitens* Newmann[1/]

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Roby and Anthony (JAVMA in press) have recently demonstrated hereditary transmission of *Babesia caballi*, the causal agent of equine piroplasmosis, in the tropical horse tick, *Dermacentor nitens* Neumann. The apparent abundance of this species on horses in areas of Florida where equine piroplasmosis is enzootic indicates that *D. nitens* may be an important vector.

Attempts were made to colonize *D. nitens* on laboratory rabbits. A horse was also infested so the life cyle on the two hosts could be compared. *Dermacentor nitens* collected from horses in Florida were supplied by the Animal Disease Eradication Division. Engorged females were allowed to oviposit in the laboratory, and 5 to 15 days after hatching the ticks were confined in muslin or linen bags cemented to the ears of rabbits and to the ear of a horse. Zippers were sewed into the ear bags so the specimens could be observed daily.

Colonization through one generation has been completed on rabbits. There appeared to be little difference in the rate of development of immature *D. nitens* when reared on rabbits or on a horse. On both hosts the larvae required an average of 7 days to complete development. The nymphs required an average of 9 days on the horse and 11 days on the rabbits. Fully engorged females first dropped from the horse 34 days after infestation. However, fully engorged females were not obtained from the rabbits until 44 days after infestation.

1/ These studies were conducted at the Beltsville Parasitological Laboratory in cooperation with the Animal Disease and Parasite Research Division.

# Laboratory Rearing of Oriental Rat Fleas and Cat Fleas

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The oriental rat flea, Xenopsylla cheopis (Rothschild), is reared on white rats in metal rearing pans. The botton of the pan is covered with a larval medium of sand and ground dog biscuits. A rat is infested with adult fleas and confined for 20 days in a small wire cage resting on the larval medium. Then, after debris has been sifted from the medium, the medium is kept in an incubator at 85° F. and 80% relative humidity. Flea pupae are separated from the medium by sifting weekly. They are kept in emergence jars until adults emerge.

The cat flea, Ctenocephalides felis felis (Bouche'), is reared very similarly to the rat flea. A flea-infested cat is kept in a cage. The cage has a removal tray underneath containing a thin layer of sand. Every 2 days, the sand is sifted to remove debris. This sand containing flea eggs is then placed in rearing pans of sand and ground dog biscuits in an incubator. The remainder of the procedure is the same as for rat fleas.

Ground dog biscuits have proven to be an adequate larval diet for these species of fleas. Powdered dry blood was found to be unnecessary as was once believed.

# Laboratory Techniques for Rearing *Heliothis* Species

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The following techniques have been used to rear the bollworm (*Heliothis zea* (Boddie)) for 18 consecutive generations and the tobacco budworm (*H. virescens* (Fabricius)) for 5 consecutive generations on a laboratory-prepared artificial diet without the introduction of outside stock. The diet used differs only slightly from that described by Vanderzant *et al.* (Jour. Econ. Ent. 55:140. 1962).

The insect eggs are collected on cheesecloth and are surface-sterilized to minimize the spread of disease by immersing the cheesecloth in 0.15% sodium hypochlorite for about 5 minutes. The hypochlorite is neutralized with sodium thiosulfate, and the cloth and eggs are rinsed with distilled water. The eggs that are washed off the cheesecloth are collected on a filter in a Buchner funnel. The wet cheesecloth is hung to drip-dry and then placed, along with the eggs on the filter, in jars covered with paper toweling. The eggs hatch in 3 to 4 days. One newly hatched larva is transferred to each 8-dram shell vial containing 15-20 g. of diet. The vials are plugged with cotton and placed in a room maintained at about 80° C. Pupae are obtained in approximately 2 weeks. The pupae are removed, washed in hypochlorite in the same manner as the eggs, and placed in 1-gallon wide-mouth glass jars on lightly moistened vermiculite. The jars are covered with cheesecloth and a strip of cheesecloth is draped into the jar so that the emerging moths can climb upon it to expand their wings. A large pioneer-size lantern globe is used for an oviposition container. The globe is set on a 6-1/2" milk filter-disk which covers moist sand in a pie pan. The moths are fed 5% sugar solution on a wad of cotton in a 50-mm. petri dish. A piece of cheesecloth is draped into the lantern globe so the moths can climb up, and the top is covered with cheesecloth also. Most of the eggs are found on the cloth over the top. A 150-mm. petri dish top is placed over the lantern globe to hold the cheescloth in place and to maintain a high humidity in the container. Under extremely dry conditions a milk filter-disk is cut to fit inside the inverted petri dish cover and wetted to provide additional moisture. High humidity appears to be essential for consistent oviposition.

Larvae for toxicological investigations are reared in 20X150 mm. petri dishes on 75-100 g. of diet that has been cut into small pieces after it has hardened. Newly hatched larvae are placed in the dish, a milk filter-disk is placed over the dish, and the cover is put in place. After 3 to 4 days, depending upon the amount of moisture condensation in the dish, the cover is removed and a masonite ring is used to hold the filter-disk cover in place. Approximately 50-75 third-instar larvae can be obtained from each dish in 5 days.

# Bollworm Rearing at College Station, Texas

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College Station, Texas

Bollworms are reared in the laboratory on an artificial media consisting of wheat germ, vitamin-free casein, sucrose, salts (W), agar, vitamins, ascorbic acid, and antimicrobial agents. Adults are kept in 1 gallon wide-mouth jars. Humidity is kept high by moistened paper towels on the bottom, and spraying with water from an aerosol kit. Food for the adults is a honey-water-vitamin solution on cotton pads. A cotton terminal and a strip of black cloth are provided for oviposition sites. Adult moths are allowed to lay eggs in each cage for 2 days, then transferred to a new cage. The eggs are allowed to hatch in the jar, and one-day larva are transferred to the media. The media for the one-day larva is poured in petri dishes, then cut up into 1/4" cubes and spread out in other dishes. About 15 to 20 larva are put in each dish, and left in 4 to 6 days. They are then transferred to individual 4 dram vials, with media poured about 1/2" deep. The larva are allowed to complete their development and pupation in these vials. They are transferred to new vials if they consume all the media or if it becomes contaminated. The pupa are put in gallon jars. The adults are removed as they emerge and put in laying cages.

# Mating Behavior in the Corn Earworm

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One of the difficulties in rearing the corn earworm involves its unpredictable mating habits. In order to insure mating, certain ecological requirements should be controlled. Since wild populations seldom attain over 50 to 60% mating, proper control of the laboratory environment is unlikely to allow for a higher than 60% mating of the reared population.

Environmental factors such as the food of the imago, light intensity, cage temperature, and humidity are all important in assuring a normal percentage of mating. These factors, along with the difficulties of rearing on harvested food, were discussed by Callahan (1962. Jour. Econ. Ent. 55(4):453-457.)

At present nothing is known about how the male and female of Heliothis zea attract each other. Methods of spatial directing of mating movements among noctuids have not been studied to any great extent. Furthermore, of our economic noctuids, little is known of how the opposite sexes persuade each other, whether by sight, sound, scent, or unknown radiation. Sexual signals and their responses often occur as a series of events dependent on a definite timing sequence. Orientation, persuasion (releasers) and synchronization are all a part of the normal mating behavior of species. Among the latter, persuasion and timing mechanisms have been overlooked in research on the noctuids.

# Rearing the Tobacco Budworm, *Heliothis virescens*

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and
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In the first experiments we tried to rear budworms on various vegetables and artificial media. Sprouted beans were a satisfactory food, but there were difficulties with fungus and canabalism. These were overcome by growing the larvae on bean plants if these were planted very thickly in a greenhouse flat, but moths did not lay well on beans. The following procedure is now in use.

Moths are held in small cages and fed sugar water. They lay eggs on small tobacco leaves that are removed from the plant and kept fresh by sticking the petiole in wet sand. Black-eye beans are heavily treated with a fungicide and planted in a mixture of soil and vermiculite in flats. When the beans are about 3 inches high they are infested with about 100 newly hatched budworms on a tobacco leaf. The flats are held in racks in lighted cages at about 85° F. By the time the larvae are about half grown they will have destroyed the beans. The flats are then interspersed with twice as many fresh ones and the budworms move over. There are then about 50 larvae per flat and these will pupate in the soil under the beans. It is essential to have the flats fairly dry when the prepupae enter them.

The bean roots form a thick mat of soil and it is necessary to remove the pupae by hand to avoid injuring them. This is a serious bottleneck which has not yet been solved.

# Artificial Diets and Rearing Techniques for the Bollworm, *Heliothis zea* Boddie

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The bollworm, *Heliothis zea* Boddie, has been reared in our laboratory for 3 years on defined diets containing casein, cysteine, sucrose, inorganic salts, corn oil, cholesterol, choline, inositol, B. vitamins, fat soluble vitamins, ascorbic acid, cellulose, agar, and water.

To prevent the destruction of the ascorbic acid and other vitamins the diet was mixed, sterilized, and cooled to 40-40° C. before the vitamins were added. The mixture then was dispersed into sterile rearing vials, 20x70 mm in size, and plugged with cotton. Bollworm eggs in the brown-ring stage were surface sterilized with a sodium hypochlorite solution and one egg placed in each vial. After growth and pupation, the pupae were removed, weighed, and put into cages for emergence. Ten generations were reared on defined diets during the winter and spring of 1961-62. Yields of adults from larvae were 70% or more.

A simple diet composed of fewer ingredients was devised for routine rearing of this insect. One of the major constituents, wheat germ, provided the only source of sterols, fatty acids and other fat soluble factors. It also provided additional protein, carbohydrate, and B vitamins.

With defined diets some of the required nutrients were determined. For good growth about 5 mg of cholesterol per 100 g of diet was needed. The fat could be placed with linoleic or linolenic acids. About 50 mg of linolenic acid per 100 g of diet was needed to promote proper emergence whereas 3 times that amount of linoleic acid was required.

Ascorbic acid was found to be an indispensable nutrient. Because this vitamin is destroyed by oxidation in the diet during the larval feeding period, analyses of insects and diets were performed to determine the changes that took place. The ascorbic acid content of both insect and diet decreased as the insect matured. It appeared that moths needed a dietary source of the vitamin to lay viable eggs.

One of the most difficult problems in rearing is to induce moths to mate and lay fertile eggs. Although good nutrition is necessary, the conditions of the physical environment seem to be the most important factors.

# Food as a Factor in Rearing Queen Bees

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To most social insects food is more than mere nourishment. It is a means of communication and a mode of life, knitting the individuals of a family into an organized social unit. In highly developed social forms such as the honey bee the sexuals have lost the function of collecting food or even feeding themselves.

The viable fertilized, and hence female-determined egg of the honey bee possesses, by heredity, the species-typical potential to develop into either of two different forms. Which of the two potentials, queen or worker, develops is determined by the food received by the bee larva. The paths, together at first do not diverge suddenly and irrevocably. A certain plasticity exists for a relatively long time to change directions by a change of food. With increasing larval age the reversion is less effective resulting in caste intermediates.

The larvae destined to become queens consume royal jelly during their entire 5-day feeding period. This food is of glandular origin produced by the pharyngeal and possibly other glands in the worker bees. It is placed in the cells in continuous and copious amounts for the developing queen larvae. This food grossly similar to milk from cows, humans, or pigeons is especially rich in certain B vitamins.

There are numerous methods of using known behavioral traits of honey bees to produce large numbers of queens in especially manipulated colonies of bees. Queens can also be reared artificially in incubators with the complete absence of adult bees. However, the food provided must be previously collected from colonies of bees. This technique has permitted a study of the components of the food. It has been shown that royal jelly is not the same during the entire feeding period and does not always produce the same results. The effects of adding or subtracting various normal ingredients is discussed.

Caste determination in honey bees remains as an unsolved problem. Certain negative information has been obtained. The future of this subject clearly lies with the chemist to discover the nature of the growth-controlling factors and to define the conditions under which they act.

# Rearing and Colonizing Wild Bees

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In terms of rearing and colonizing them, there are three distinct types of wild bees: Those that construct colonial nests in large cavities (bumble bees), those that dig burrows in the soil (andrenids, halictids, and anthophorids), and those that fit their brood cells into pre-existing cavities (megachilids, xylocopids, and hylaeines).

Bumble bee queens can be collected in the spring and confined to domiciles or to enclosures containing domiciles. Success with each method varies according to species. Domiciles can be placed in the field for acceptance by queens. Proper placement; i.e., above ground, on the surface, underground, etc., varies according to species. Colonies should not be kept close to one another. They can be fed honey and pollen but this tends to inhibit the workers from foraging in the field. Queens will hibernate in the greenhouse in piles of loose soil covered with pine needles. Individual rearing of larvae has not been tried.

Gregarious species of soil-burrowing bees can be induced to colonize soil areas specially prepared to meet their requirements. "Artificial" nesting sites for alkali bees (Nomia melanderi Ckll.), now commonly constructed by alfalfa seed growers, consist essentially of a basin lined with plastic film and filled with soil. A layer of gravel is placed above the film to transport water which is added through pipes extending from the soil surface to the gravel layer. A constant level of soil moisture can be thus maintained. Most soil-burrowing species brought into a greenhouse provided with suitable forage and soil will nest therein, especially if introduced before they have established nests in the field. They usually accept honey but not pollen placed in the flowers on which they forage. Individual larvae can be raised from eggs left on their original pollen mass. Great care must be taken to maintain the pollen mass in its original state of moisture and to prevent its invasion by microorganisms. Small plaster-of-Paris cups covered with dialyzing membrane have been used successfully as rearing chambers. It is extremely difficult to move eggs and young larvae to a new position on the food mass or to a new food mass.

Many species of megachilids and hylaeines accept holes provided for them in their natural nesting areas. These also nest satisfactorily in the greenhouse if provided with forage and suitable nesting holes. Wood blocks drilled with holes, drinking straws, and corrugated cardboard are examples of nesting materials accepted by various species. Individual larvae are much easier to rear than are those of soil-burrowing species. The eggs and young larvae of megachilids, in particular, are more "rugged" than those of other bees and can easily be moved to new food masses. One species, Osmia lignaria, has even been partially reared on "unnatural" diets.

# Rearing Honey Bee Larvae

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A method of rearing honey bee larvae under controlled laboratory conditions has been devised by utilizing a self-feeding dish. A pad of absorbent cotton saturated with a liquid food is placed in a petri dish and covered with plastic screening. The larvae rest upon the plastic screening and feed through the interstices or wells of the screening. When the larvae cease feeding, they are transferred to petri dishes containing a thin layer of beeswax on the bottom, where they spin a pad of silk, pupate normally and emerge as adults.

By utilizing this method disease or development studies can be made in a continuous manner on a daily or hourly basis. Any stage or age of larva or pupa and the complete development cycle from larvae 3 days old through to the adult bee are made available for experimental observation.

Controlled feeding is also accomplished by using a Dutky-Fest microinjector and a 30-gauge needle. Oral introductions of material are made directly into the midgut. Honey bee larvae weighing as little as 25 milligrams can be fed by this technique.

# Importation of Immature Stages of Honey Bees

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Two general methods have been employed with considerable success. (1) Sperm from the desired race or strain of bees is collected from drones and placed in a melting point tube. About 1.2 µl of semen can be collected from each drone and 30 to 40 µl of semen are placed in each sealed melting point tube. The tubes of semen are then protected from breakage and sent by ordinary air mail. By three generations and three such inseminations stock will be over 85% of the desired genetic trait.

(2) Eggs, young larvae and queen pupae of the desired stock have been imported by means of a portable incubator maintained at brood rearing temperature of 34° C. transported as personal luggage. Eggs are timed to hatch on arrival at the destination. Larvae are fed royal jelly enroute at 24-hour intervals and queen pupae are placed in gelatin capsules. Importation shipments of an unaccompanied incubator have been unsuccessful as have refrigerated shipments of the immature forms.

# Rearing Codling Moth in the Laboratory

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Yakima, Washington

These methods are used at the Yakima station where all phases of rearing are conducted in chambers at 80° F. and 60% relative humidity. Eggs are obtained on wax paper liners in plastic cages 5" in diameter and 11" high with 1/2 gallon ice cream carton tops for the ends of the cage. There are two 1/2" holes in the tops, one with a stopper provides for the introduction of moths and one covered with 16-mesh screen provides ventilation. The wax paper is cut and glued to form a cylindrical liner, the ends of which are slit, turned outward and backward, and held in place under the ice cream carton tops. Water is provided in a ball of damp cotton. This cage will accommodate 100-125 moths (both sexes) and will provide for the collection of 1,500 eggs per day. Moths live about 14 days. The female begins deposition within one day after emergence, and deposits about 200 eggs, 90% within 5-6 days. Oviposition is induced by use of a 1-4 C.P. 7-watt red electric light or white light covered with red cellophane.

Eggs on small pieces of paper applied to small green apples in wooden trays at the rate of about 3 eggs (blackspotted and ready to hatch) per apple produce about 1.3 mature larvae (and adults) per apple. Larvae form cocoons in corrugated (4-5 corrugations per inch) cardboard strips one-half inch wide attached to the sides of the trays or in strips placed on apples. The development of larvae and pupae to the beginning of moth emergence requires 24-25 days.

A wooden tray 15"x15"x4" with a muslin cloth and frame top is used to hold the infested apples. The tray top may be eliminated by use of hot wire or electrically charged metal strips 1/16-1/8" apart on the sides of trays to prevent escape of mature larvae seeking cocooning sites. Continuous light from a 7-watt lamp above each tray prevents diapause of the larvae.

Immature apples about 1-1/4" in diameter are obtained during late June or early July when trees are usually thinned to reduce fruit load. Growers will pick the apples for $1.00 to $1.50 per apple box (42 lb.) and each box contains from 800-1,000 apples. These apples can be stored at 32° F. for about a year in polyethylene-lined apple boxes with a layer of shreaded newspaper below and above the apples to absorb surplus water. One-half inch slits in the polyethylene bag near each corner of the box provide openings for air-gas exchange and prevent apple suffocation and spoilage. Apples are soaked 10-15 minutes in a trisodium phosphate solution (1 lb. to 6 gallons of water) to neutralize toxic insecticides before storage. Apples sprayed with Guthion should not be used because this material is absorbed by the skin of the fruits and cannot be neutralized with trisodium phosphate or removed satisfactorily with solvents.

# Concentrate Media for Codling Moth and Red-banded Leaf Roller

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Vincennes, Indiana

A program for development of concentrate media for rearing the codling moth (Carpocapsa pomonella (L.)) and the red-banded leaf roller (Argyrotaenia velutinana (Walker)) was initiated in December 1960 to provide specimens throughout the year for biological studies, for screening insecticides, for evaluating chemosterilants, for studying sex attractants, and for maintaining and studying insecticide resistant strains. This program will obviate the need to collect hibernating larvae in the fall of the year for holding in cold storage until needed, as in the past.

The most efficient formula for rearing the codling moth contained apple seeds 6.0 gms., dried apple 12.0 gms., English walnut meats 12.0 gms., sugar 12.0 gms., soybean protein 12.0 gms., Wessons salt "W" 2.5 gms., alphacel 6.0 gms., glycine 0.125 gms., cysteine 0.25 gms., cholesterol 0.125 gms., agar 4.0 gms., KOH (10% solution) 7.0 ml., vitamin solution 2.5 ml., mold inhibitor 4.5 ml., water 220.0 ml., and ascorbic acid 1.2 gms. (dissolved in water).

The optimum rate of ascorbic acid needed in the formula has not been established but recent results indicate it to be between 0.4 and 0.9%.

To prepare the formula the dried apple, apple seeds, English walnut meats, and 120-130 ml. of boiling water are blended together in a blender. After the mixture reaches a paste consistency, a boiling water-agar solution (100 ml.) is added. Next, all other dry ingredients, except alphacel, and the remaining liquid ingredients, except ascorbic acid, are blended into the mixture. Then, the alphacel is added, the medium cooled to 140° F., and the ascorbic acid blended into it. The medium is dispensed into jelly cups by means of a plastic squeeze bottle, using about 1/4 ounce per cup. Newly hatched codling moth larvae, two per cup, are transferred to the medium with a camel's hair brush.

A simplified medium developed for rearing the red-banded leaf roller at Vincennes contains 62.5 gms. alfalfa leaf meal, 5 gms. agar, 13 ml. mold inhibitor, 6 ml. vitamin solution, and 500 ml. of water. Its preparation is similar to that described for the codling moth medium. After the medium in jelly cups has hardened, it is scored with a knife before larvae are placed on it. Approximately 75% of the larvae emerge as adults.

An earlier but more complicated formula that was found to be satisfactory for red-banded leaf roller rearing will be published in the April 1963 issue of the Journal of Economic Entomology.

# Nutrition and Rearing of the Plum Curculio

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and
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Fruit and Vegetable Insects Research Branch
Fort Valley, Georgia

Nutrition: This insect can be reared without difficulty on small green apples about an inch in diameter. We get a year's supply of such apples in early June and store them until needed in a walk-in refrigerator maintained at about 34° F. The secret of using and having available this food throughout the year is to pick only good fruits and keep them in storage as described. Do not use thinned fruits that have been thrown to the ground or fruits that have fallen under the trees. Such fruit will not carry from one season to another. The apples are thoroughly washed before use.

Rearing: Rearing is carried on at a constant temperature of 80° ± 1° F. and a constant humidity of 60 ± 1%. Two complete compressor units are rigged up so that one will automatically take over should the other "conk" out. Furthermore, the heating apparatus has a safety device so that it will shut off should something go wrong before the temperature rises to a point where the insects will be killed. We have reared one generation after another, throughout the year for five years with no evidence of any diapause.

A specially constructed cage with sheet metal frame and screen-wire top, sides, and bottom is used for oviposition. It is divided with hardware cloth to provide space for apples for oviposition in the top half and space into which to jar the insects to prevent interference when handling the apples maintained for oviposition in the top half. A bottle of water stoppered with a cotton dental roll and protruding from the neck of the bottle is placed in the top half of the cage with the apples. About 30 adults are put in each cage, and the apples in which the eggs have been deposited are removed every other day.

The apples containing the eggs are removed to larval emergence boxes with a hardware cloth bottom. The emerging larvae can be collected from trays placed beneath the boxes or allowed to go directly into the soil for pupation by placing a box containing soil beneath the larval emergence box. The soil in the pupation boxes should be watered every other day. After having spent about a month in the soil as larva, pupa, and adult, the adult emerges and is collected from the pupation box. The pupation boxes are equipped with wire screen tops padded with felt. Around 50 days are required for the insect to complete its entire life cycle.

1/ Presented by Julius P. Hollon.

# The Technique Used in Rearing Mexican Fruit Fly, *Anastrepha ludens* (Loew), in the Laboratory

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Before emergence puparia are placed inside cages of 2'x3'x4' which hold 20,000 flies without crowding. Food of sugar, orange juice crystals, and protein hydrolysate and water is placed in the cages. Beginning 15 days after emergence flies are egged three times weekly using moistened oviposition shells exposed for 2-1/2 hours. The eggs are then washed out, measured, and deposited on 3-1/2" organdy discs in petri dishes. Approximately 6,000 eggs, or 0.3 cc., are placed on each disc previously moistened with 0.07% sodium benzoate solution to inhibit fungus growth. The eggs are incubated for 4 days at 82° F. and 80% R. H.

The larval medium is prepared using either fresh ground or dehydrated granulated carrots. To this base is added 4% NBC Brewer's Yeast, 0.08% sodium benzoate, 0.1% methyl-p-hydroxybenzoate, 0.3% enzymatic yeast hydrolysate and hydrochloric acid to adjust to a pH of 3.5. With dehydrated granulated carrots 6 liters of water are added to each 775 grams. The ingredients are mixed thoroughly and placed in plastic lined trays 6 liters per tray. Approximately 30,000 eggs are placed on each tray, the trays are covered and held for 10 days at 80° F. and 80% R. H.

Mature larvae are removed by straining the medium diluted with water. The larvae are measured volumetrically (16 larvae = 1 cc.) and placed on moistened sand and finely ground vermiculite in equal proportions not over 3 to 4 per cc. The samples of larvae taken at random from each rearing are weighed. The pupae are kept for 15-16 days at 82° F. and 80% R. H. and then screened out.

Vitamins, amino acids, and other materials are added to the carrot medium to determine if larval recovery can be improved.

# Laboratory Methods for Rearing Rust Mites on Citrus

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and
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Two species of rust mites have been found on citrus in Florida, the citrus rust mite, Phyllocoptruta oleivora (Ashmead), and Aculus pelekassi Keifer. The latter was first found in laboratory colonies of rust mites being reared on citrus seedlings at Orlando.

A method for rearing rust mites has been developed in which large populations of mites are maintained on murcott honey orange seedlings during all months of the year. Plants are kept in 52 mesh lumite screen cages with mylar tops. Before infestation the caged plants are kept in insectaries. Infested plants are kept in air-conditioned greenhouses maintained at 76-82° F. with humidity ranging from 30 to 60% R. H. during the day and up to 95% at night during summer months. A technique was developed for isolating or confining rust mites within open cages consisting of a ring of lanolin on citrus leaves or fruit.

Mites are transferred by brushing from an infested plant to a clean plant or fruit using a camel's hair brush, by cutting infested leaves into narrow strips that are placed on a clean plant, or by moving individual or groups of mites on a single hair or bristle in a needle holder. The third method is generally used and is facilitated by using a wide field "zoom" microscope mounted on a rotatable arm. Preliminary investigations have shown that the optimum temperature for rearing rust mites is about 80° F., at which they go through their life cycle in 6-7 days. Humidities below 35% R. H. are detrimental to rust mites but at sustained high humidities the mites are attacked by a fungus disease, Hirsutella thompsonii Fisher. Differences in day length also affect rust mites.

Phyllocoptruta oleivora is generally yellow in color and may be found on "hardened off" leaves, green twigs, or fruit. They cause a rusty appearance of fruit and may cause firing of leaves. Aculus pelekassi is pink or yellow in color and may be found on immature as well as mature leaves. They cause severe distortion of immature leaves. No information is available on damage to fruit. The two species may be differentiated only under a compound microscope. A. pelekassi has shown a positive response to fluorescent light and generally is much more active than P. oleivora. Studies are underway to evaluate the effects of chemicals and sterilants on both species of mites. Biological studies of both species also are being conducted along with studies of biological control using fungus diseases of the mites.

1/ Presented by A. K. Burditt.

# Laboratory Rearing Techniques for California Red Scale and Citrus Red Mite

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California red scale and citrus red mite are both reared on green lemons in the laboratory. At 77° F. and 70% R. H., red scale completes its life cycle in 42 to 45 days. New infestations for propagating the scales are made by attaching frustums made of heavy paper to the old reservoir lemons and placing the new lemons on top of the frustums. The crawlers then migrate over the frustums to the fresh lemons. For experimental purposes, lemons infested with scale are held on Henderson racks, where they can be maintained satisfactorily for up to 6 months.

At 75° F. and 60% R. H. the citrus red mite completes its life cycle in about 14 days. For routine propagation, mites are reared in isolation boxes in units of 10 lemons each. In starting new cultures, two methods of mite transfer are employed: (1) Self transfer, by placing fresh lemons on rubber collars which have been set on the host lemons bearing the mites, and (2) brushing the mites from the host lemons onto a unit of fresh lemons. Oviposition on the new lemons is limited to 2 to 4 days to maintain distinct generations of mites of nearly uniform age.

In studies of resistance to different acaricides, mites may be held on treated lemons placed in plastic funnels and partially immersed in water to form a moat which restricts the mites to the lemons during the test. In another method, lemons upon which mites have oviposited are treated and the surface of the lemons bearing the eggs sealed in cages. The mites are then under continuous exposure to the chemical during the development to maturity.

In studies of a viruslike disease of the citrus red mite, the mites are mass reared on lemons held on wooden frames which have wire grids stretched across to support the lemons. Mass transfer of mites from one frame to another is facilitated by using an air jet in an infesting tower. After the mites on a frame have developed to the protonymph stage, they are inoculated with a spray suspension of the pathogen and held until they become diseased and can be collected for experimental work.

In attempts to develop an artificial diet, mites have demonstrated the ability to feed through certain membranes on nutrient solutions and on agar disks containing nutrients. However, several mechanical problems remain to be solved before extended studies of artificial media can be made.

# Rearing Green Peach Aphids

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Beltsville, Maryland

A culture of the green peach aphid, Myzus persicae (Sulzer), has been maintained since 1938 in the greenhouse during the winter and the insectary in summer on collards or other crucifers. These aphids are used in virus vector tests, insecticide screening, and testing canisters and filters in respiratory equipment.

When temperatures exceed 85° F. the aphids are moved to cloth covered cages in the insectary. Vigorous young plants are supplied liquid nitrogenous fertilizer and are watered regularly to avert wilting. If aphids are not used in tests the colonies are reduced and supplied fresh plants to reduce the tendency for production of winged aphids.

When more than one species or strain of aphid is maintained, differential hosts are selected in addition to isolation of colonies in separate greenhouse units.

Automatic controls for heating and ventilating equipment, lighting to regulate host plant growth, and watering devices have been utilized to provide better growing conditions and to reduce the need for personal attendance.

# Rearing the Predaceous Mites, Phytoseiulus persimilis A. H. and Typhlodromus fallacis (Garman)

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Colonies of predaceous mites, Typhlodromus fallacis (Garman), occurring locally, and Phytoseiulus persimilis A. H., from West Germany, were reared in electrically heated and lighted chambers. As food they were supplied the spider mites, Tetranychus telarius (L.) and T. cinnabarinus (Boisd.), on lima beans. Heavily infested plants were placed at weekly intervals in the chamber and adult predator mites quickly moved to the fresh food supplies. Older plants vacated by predators after having destroyed the Tetranychids were removed. Immature persimilis consumed 6-7 eggs and adults devoured 18-20 eggs plus 1.5 adult female Tetranychus per day.

Maximum production was about 500 predator mites per week which were taken from the chamber for release or pesticide residue tests.

Artificial diets have not been investigated but acceptable host mites appear to be rather limited. Preliminary studies indicated that mites could be stockpiled for short periods at cool temperatures, but above 40° F. adults and eggs held at 31° and 40° F. for one week were killed.

# Mass Rearing of Drosophila melanogaster

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In 1962, 3.24 million Drosophila melanogaster adults were reared and sexed, and the apholate-treated males were released in infested tomato fields for testing the chemosterilant method of control. A cornmeal-agar-sirup-yeast rearing medium (Fleming, USDA Tech. Bul. No. 1266, 1962) was used in greenhouse flats lined with removable aluminum foil and covered with cheesecloth and polyethylene. A maximum of 78 thousand flies were reared per unit but larger, more uniform flies were obtained by less crowding which resulted in an average yield of 23,500 flies per flat. The cost of rearing medium and liner was $1.40 per flat or about 6 cents per 1,000 flies.

Covered flats with cooled media and 2500 breeder flies in each were placed in front of fans on shelves in a modified mushroom house with walls covered with polyethylene to reduce moisture penetration. A combination room-air-conditioner and humidity-control unit maintained 45% R. H. and 72-75° F. in the rearing room.

Excessive moisture conditions in the flats that coincided with activity of maturing larvae were controlled by raising the polyethylene cover to permit increased evaporation.

Adult flies were removed from flats by attracting them toward a light into a cloth and plastic covered 2'x2'x3' chamber located in a darkened room. Flies were collected from the chamber into gallon jars by means of a vacuum cleaner, then etherized and sexed in a laboratory constructed unit that provided an upward moving current of air in a glass tower. The lighter males were carried higher in the tower and were taken into a collecting jar by the air current. Lots of 20 thousand males in screen cages after recovering from the ether were fed overnight on a sugar-yeast mixture containing apholate then released in the tomato fields. To identify released flies in the field they were sprayed with rhodamine-B just prior to release.

# Techniques Used in Rearing the Cabbage Looper, The Banded Cucumber Beetle, The Southern Potato Wireworm, and a Dipterous Leafminer

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Four species of insects are reared at Charleston for an insecticide screening program. Cultures of the cabbage looper, Trichoplusia ni (Hubner), and the banded cucumber beetle, Diabrotica balteata LeConte, are maintained year round. The southern potato wireworm, Conoderus falli Lane, and the leafminer, Liriomyza sp., are reared only during the summer.

## The Cabbage Looper

A method described by F. L. McEwen and G. E. R. Hervey (1960. Ann. Ent. Soc. Amer. 53:229-34) is used with a few minor modifications. The main differences involve increased sanitation measures to avoid diseases. All foliage used is soaked for 15 minutes in a 0.5% sodium hypochlorite solution. A semi-artificial media containing collard powder, formaldehyde, methyl parahydrozybenzoate, ascorbic acid, agar, and water has shown promise as a larval diet.

## The Banded Cucumber Beetle

Adults are confined in glass fruit jars equipped with a screen wire top and fed on sweetpotato or collard foliage. To obtain eggs the jars are inverted and the tops pressed down into dampened peat. Larvae are reared in damp peat in plastic food crispers and fed on sprouting corn and sweetpotato roots.

## The Southern Potato Wireworm

A method described by Cuthbert (1962. Jour. Econ. Ent. 55:262-3) is being used.

## A Dipterous Leafminer

Adults are confined in a muslin-covered cage containing potted cowpea seedlings for food and oviposition. Larvae mature in the cowpea foliage and are allowed to pupate in small plastic containers. Emerging adults are trapped in an inverted glass flask and any parasites present removed.

# Two-spotted Spider Mite Rearing and Procedures

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Organophosphate resistant and non-resistant two-spotted spider mites, Tetranychus telarius (L.), and various selections from the present colonies have been reared for experimental purposes for about 15 years at Beltsville, Maryland.

The nature of the studies on biology, genetics, and the screening program for comparative purposes demand strict isolation so that each strain is distinct.

Isolation is accomplished by rearing in separate greenhouse ranges or compartments within a range. Careful attention to clean uninfested feed plants supplied to the colonies on a definite schedule is stressed.

Methods of mite handling, transfer, and treatment of immature stages and adults are discussed.

# Studies of Mass Rearing of the Tobacco Hornworm

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and
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In the first experiment in this series an attempt was made to rear 3,000 hornworms by collecting eggs in the field (because moths were not available), and feeding the larvae on cut tobacco plants set in buckets of water on a sheet of plastic. Fully grown larvae crawled to the floor and were picked up each morning and placed in 1 quart, paper, milk cartons half filled with sand. About 1500 pupae were recovered.

In the second experiment moths laid an estimated 21,000 eggs in a large outdoor cage on growing tobacco plants. The larvae grew to the last instar and destroyed the plants. They were then collected in buckets and placed on a floor with fresh tobacco. Prepupae were again placed in milk cartons in sand or sawdust. The field cage was highly successful and 18,000 fifth instar larvae were collected, but the tobacco on the floor became mixed with feces and spoiled. Most of the larvae died and only 5,000 pupae were recovered. Sawdust proved satisfactory for pupation and was much lighter and easier to handle than sand.

In the third experiment the tobacco stalks in the field cage were cut off and a crop of suckers allowed to grow. Hornworms were again reared in the same way but removed in the fourth and fifth instars, placed on fresh tobacco hung on racks over tables covered with hardware cloth. In this way larvae could feed, but the feces fell through the screen. Prepupae crawled off the table into a box and pupated in milk cartons or in boxes 1'x3'. About 20,000 were recovered in this way.

Further improvements are needed to reduce the space required for pupation, and reduce the labor needed to handle tobacco and larvae.

1/ Presented by F. R. Lawson.

# The Tobacco Hornworm

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and
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Fruit and Vegetable Insects Research Branch
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This is not a report on rearing techniques, but rather where we let the tobacco growers help us rear the insects.

A sex attractant for the male tobacco hornworm was recovered at Florence in 1961. Since then we have needed large numbers of virgin females to get material for the chemist to determine what the attractant is as well as for field testing.

During the latter part of the 1962 season, large hornworm larvae were collected from tobacco plants or plant suckers on growers' farms, transported to the Experiment Station, placed on suckers that had been collected on the grower's farm, and allowed to pupate in the soil.

Approximately 100,000 large larvae were collected. They were crowded into 15 pupation bins and a little over 33,000 living pupae recovered from the soil. The pupae were cleaned to remove the soil, sexed, and placed in cardboard boxes that contained strips of newspaper. From 500 to 600 pupae were placed in each box of paper and suspended in a quonset hut by means of two strands of hay baling wire. The boxes had to be kept above the floor of the quonset hut to keep rats from destroying the pupae. The boxes containing the pupae have been in the unheated quonset hut throughout the winter despite a minimum temperature of 9° F. Around the first of May they will be taken from the quonset hut and placed in a walk-in refrigerator to keep them from emerging in large numbers in a short period of time. The female pupae will be taken from the refrigerator in lots of about 200 per day and allowed to emerge in a normal manner.

1/ Presented by Norman Allen.

# Propagating *Tiphia vernalis*, A Parasite of Japanese Beetle Larvae

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During the years when insect parasites were imported and propagated for release against the Japanese beetle a method for the mass propagation of *Tiphia vernalis*, a parasite of Japanese beetle larvae, was evolved in the laboratory.

Laboratory oviposition was obtained by using either field collected or laboratory reared female *Tiphia* in compartmented individual oviposition cans of soil containing six carefully dug host grubs. The oviposition cans were emptied daily to permit removal of parasitized grubs and the transfer of the parasite to a fresh oviposition unit. Records showed that females collected in the field early in the flight period were 40% more productive than laboratory reared stock.

Parasitized grubs thus obtained were stored at 75° F. in individual compartments within specially constructed flats filled with seeded soil. When the parasite larvae had formed their cocoons about four weeks later, the contents of the cocoon formation flats were screened and the cocoons transferred to individual glass vials. The trays of vials were overwintered at field temperatures in controlled temperature hibernation chambers.

The following spring the vials were stoppered with screen caps and as emergence of adult *Tiphia* occurred they were collected daily and released in a frame and muslin hood for observed mating. Mated females were used for further propagation or for field release.

It was estimated that 12,000 female *T. vernalis* could be produced with a propagation program involving 500,000 host grubs and 4,000 gravid female *Tiphia*. Experiments with multiple generation propagation proved such a procedure to be impracticable because the parasites did not react favorably to forced development.

Feeding tests with 12 foods or combinations of foods indicated that there was no important difference in the results obtained between any of them and those obtained when 30% honey solution was used.

# Rearing Entomophagous Insects

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Although the Moorestown, New Jersey, Laboratory does not rear large quantities of parasites, it does propagate and ship a wide variety of species, and it has developed a number of rather simple tools which have proved very valuable for this purpose. In rearing the rice moth as a host of pink bollworm parasites, it was found that rice moth eggs could be readily separated from moth scales, if eggs were oviposited over aluminum foil. Rearing Apanteles angaleti, a parasite of the pink bollworm was greatly facilitated by obtaining oviposition on host larvae held in small glass containers covered with cheese cloth. Similar containers were used to rear egg parasites, alfalfa weevil, and corn borer parasites. When plants are required, a bottomless cage which can be set over infested plants is used. Sides and top are of saran cloth stapled to wooden frames, the sliding door is plexiglass and the cage sets on a gasket of polyurethane. All parts are made separately and securely screwed together to insure tight joints. A standard pint food container with a circular opening cut into the lid, that can be closed with a milk cap, having a similar screened opening in the bottom, is the standard container used for shipping. Insects are usually drawn into these containers with air suction. Moisture is supplied on a cellulose sponge held in place by an additional lid covering the bottom. This type of container has recently been successfully used to ship Coccenellids from Barbados to Hawaii via Moorestown.

# Colonization of Two Hymenopterous Parasites (Family Pteromalidae) of Muscoid Pupae

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Laboratory colonies of Spalangia muscidarum Rich. and Muscidifurax raptor G. & S. were established in 1958 and 1959, respectively, from specimens collected in the vicinity of Lincoln, Nebraska. Colonies have been maintained continuously to the present. House fly and stable fly pupae, one to three days old, serve equally well as hosts. Both species of parasites can be reared by the same methods. For small colonies the hosts are reared by standard procedures. When pupation begins the adult parasites are placed in the container with the hosts. The container is covered with a cloth held in place with a rubber band. The parasitized pupae are separated from the medium just prior to or following emergence of flies that escaped parasitism. They are cleaned, dried, and stored in a suitable place until the adult parasites emerge. The procedure used in mass rearing the parasites is changed slightly. The adult parasites are liberated in a room large enough to accommodate the tubs in which the hosts are reared. When the fly larvae begin to pupate the rearing containers are set into the room with the parasites. After three days, the containers are removed and the puparia are separated from the medium. The life cycles of S. muscidarum and M. raptor are approximately 3 and 2-1/2 weeks, respectively, when reared at a temperature of 80 to 85° F.

# Rearing Parasites of the Sugarcane Borer

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Parasite rearing at the Canal Point, Florida, Laboratory at present consists of three phases: (1) Exploratory studies to determine whether parasite species that host on Diatraea spp. abroad can reproduce on the sugarcane borer, Diatraea saccharalis; (2) Adapt or modify existing rearing methods or devise new ones which will abet culturing sufficient numbers of parasites for study of biology, etc. relevant to parasitism of the new host; (3) Develop methods whereby the parasite can be mass reared whenever results of laboratory studies indicate field releases are practical.

The ultimate purpose of laboratory rearing is to obtain species in the field which will assist in natural control of the pest.

Discussion will be limited almost in the entirety to Apanteles flavipes, a Hymenopterous parasite acquired from India as a result of the P. L. 480 bill.

Parasitism of field collected host larvae (late instar sugarcane borer), contained in 1-1/2 to 2" sections of 1/8" diameter plastic sipping straws, is accomplished by introducing a single female parasite into the straw and allowing it to oviposit only once. The females are collected for further use as they crawl from the straws after ovipositing and the host larvae are blown from the straws individually into vials containing a natural food source. These infested host larvae are held at 78 to 82° F. for 16 days. During this period 20 to 80 parasites per individual develop in about 40% of the parasitized hosts, emerge and spin cocoons.

Larval maturity of the parasite is usually attained in 12 to 15 days. Duration of the pupal and adult stages is approximately 5 days each.

Egg oviposition by an individual female, under laboratory conditions generally occurs 3 to 5 times, often resulting in as many as 5 hosts parasitized by a single female. Offspring of the fertilized females consists of about 80% females. Parthenogenetic reproduction results in all male progeny.

The parasite has been reared through 9 generations using the above methods and it is anticipated field releases will be made during April or May of this year (1963).

# Rearing Insects for Weed Control

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The requirement of an insect for nourishment from its preferred host plants in order to induce oogenesis has been demonstrated for only a few weevils. Similar studies are being continued because of their importance to the problem of host plant specificity, and -- in the case of those species whose larvae feed upon the seeds and fruits of plants -- the synchronization of oviposition and subsequent larval development with seed or fruit maturation.

D. M. Maddox, in working with the imported gorse seed weevil, Apion ulicis Forst., determined that the weevils in the field feed upon gorse, Ulex europaeus L., year-round but only matured sexually after feeding on the bloom of gorse. In laboratory tests, feeding only on gorse foliage initiated oogenesis in 7% of the females, while foliage plus stamens only produced 13% development. Foliage plus corolla only or foliage plus complete flowers provided 94% maturity in both series. He concluded that the corolla of the gorse flower plays a vital part in the oogenesis of the gorse seed weevil.

G. W. Angalet and L. A. Andres proved that the two species of puncture vine weevils were dependent upon the nutrition obtained from their preferred host plant, puncture vine, Tribulus terrestris L., for continued egg maturation. One species, Microlarinus lypriformis (Woll.), has stem-feeding larvae while the other, M. lareynii (Duv.), has seed-eating larvae. The adults of both species will feed on many plants to sustain life but oogenesis occurred only when puncture vine was provided. When it was withdrawn, ooabsorption took place and egg production commenced again only after puncture vine was again supplied. Two closely related plants either did not furnish sufficient nourishment for oogenesis, as two species of caltrop, Kallstroemia spp., or for limited, irregular egg development, as a beancaper, Zygophyllum fabago L.

# A Technique for Mass Rearing of the Greater Wax Moth and the DD-136 Nematode

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An 8 mm. film prepared by Dr. A. M. Heimpel and his staff was shown to illustrate the procedures used in the mass rearing of the greater wax moth and the DD-136 nematode.

The technique employed for the greater wax moth, Galleria mellonella, has been published, (1962. Proceedings, Ent. Soc. Wash. Vol. 64, No. 1 56-58) and reprints of this paper are available for distribution.

A manuscript giving the essential techniques of the nematode rearing, harvest, and storage has been prepared and approved by the Publications Officer, and will be submitted for publication shortly.

# Mass Rearing Cabbage Loopers on a Semisynthetic Diet and Propagation of the Nuclear Polyhedrosis

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Rearing Technique: Previously, laboratory studies on the cabbage looper, Trichoplusia ni (Hubner), were curtailed due to difficulties encountered in excluding the polyhedrosis disease or in obtaining natural food. This study describes a synthetic diet, apparatus, and procedures which were successfully used for over 30 generations (average generation 23.0 ± 0.6 days). The looper culture has been maintained continuously for over 40 generations. The synthetic diet of the pink bollworm developed by Vanderzant and Reiser (1956. Jour. Econ. Ent. 49:7-10), modified by Adkisson et al. (1960. Jour. Econ. Ent. 53:759-62), Clark et al (1961. Jour. Econ. Ent. 54:4-9), and Ouye (1962. Jour. Econ. Ent. 55:854-7), was fortified with dried, powdered cotton leaves, formaldehyde, and aureomycin. Methycellulose, freeze-dry preparations of cabbage, lettuce, parsley, broccoli, and cotton leaves were also substituted for the dried cotton leaves. The rearing technique is summarized as follows: Eggs were collected on paper toweling wrapped around 1/4-inch mesh, screen oviposition cages. Paper toweling with the attached eggs were then placed in a porcelain pan and soaked for 5 minutes in 0.3% sodium hypochlorite. The hypochlorite freed the eggs from the paper by exchorination and also eliminated polyhedral contamination (Ignoffo & Dutky, unpublished). The hypochlorite-egg suspension was filtered by suction rinsed with sterile 10% sodium thiosulfite and then washed several times with sterile, distilled water. The filter paper with the eggs was then transferred to a Petri dish containing medium, plugged with cotton batting, and incubated at 29° C. for 3-4 days. Thirteen 1st to 2nd instar larvae were transferred from the Petri dishes to a 1/2-pint, wide mouth, glass rearing jar using a sterile camel's hair brush. The rearing jars were covered with bolting cloth, dated, and incubated at 29° C. Approximately, 9 days later pupae were collected and placed in quart size ice cream cartons for adult emergence. The adults were then immobilized with $CO_2$ and placed in the screen oviposition cages.

Propagation of Polyhedra: A simple technique was devised for infecting the larvae with the polyhedrosis. Larvae reared in jars at 28 ± 1° C. for 7 days (late fourth and early fifth instar) were used to propagate polyhedra. Both the larvae and diet were sprayed with 0.5 ml. of a suspension containing 5 X $10^6$ polyhedra/ml. using a hand atomizer. The treated jars were then placed at 23° C. This temperature (23° C.) permitted harvesting of diseased, living, intact larvae. Diseased, late fifth-instar larvae were harvested 4 to 5 days after spraying and stored at -30° C. Polyhedral suspensions, standardized as to count and virulence, were used for subsequent studies and also made available to other workers.

# Control of Diseases in Insect Rearing

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In insect rearing, control of diseases is vital to a successful program. Selection of clean disease-free rearing stocks and careful sanitation are essential to maintain continuous culture of insects in large numbers. The procedures used should be constantly reviewed to determine where breaches in security might occur and how these can be circumvented.

Where possible, cultures should be infested with eggs since at this stage most disease organisms and non-pathogenic contaminants that might compete nutritionally with the insects can be eliminated by surface sterilization and the treated eggs can then be safely introduced into the rearing medium by simple aseptic or antiseptic procedures. Sodium hypochlorite 0.1% aqueous solution is an effective surface sterilant against many pathogens including viruses, fungi, and bacterial spores.

Partial dechorionation of the eggs may result from this treatment and require modification of techniques used successfully with untreated eggs to prevent dehydration of treated eggs before hatch. This effect is particularly important with those insects that are reared in dry media or in media of high osmotic pressures- as is the case in stored-products insects.

Insects reared on living plant tissues require somewhat less attention to elimination of non-specific microorganisms than do insects reared on artificial media, and most attention can be directed toward elimination of specific insect pathogens that might be introduced into the cultures along with the food plants. Immersing the food plants or foliage in sodium hypochlorite 0.5% aqueous solution prior to use is helpful in eliminating this source of contamination of cultures. Where insects are reared on artificial diets, completely aseptic or antiseptic procedures are essential to safeguard cultures since the suppressive systems that prevent growth of non-specific contaminant in living tissues are not active in the artificial diets.

Microbial antibiotics and antimetabolites are of great value in insect control. They can be used to obtain disease-free rearing stocks from populations with a high disease incidence when this cannot readily be done by selection alone. For this purpose, the antibiotic can usually be best administered by microinjection. For many bacterial diseases, 20 micrograms of dihydrostreptomycin per insect is a satisfactory dosage. Proper selection of the antibiotic or antimetabolite can be made by employing antibiotic sensitivity discs. The addition of antibiotics to mass rearing media may also be effective, but this is not recommended as a routine practice since it may result in the selection of resistant strains of the pathogens that will be extremely troublesome in the long run. Continued use of antiseptics or antimetabolites added to mass rearing media also can produce the same result.

# Citrus Insect Parasites and Predators

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Lake Alfred, Florida

Rearing predators and parasites of citrus insects at Lake Alfred has been confined largely to limited rearing for life history, food preference, and other similar studies. Rearing equipment has been simple. Fruit and seedling citrus trees have been used for host material to rear the citrus pests. Current efforts are directed towards evaluating possible spider mite predators and pathogens and finding and evaluating predators or pathogens for rust mite control. Recent successful introductions for scale control (one accidental or natural for purple scale, the other, an intentional effort for Florida red scale) have given excellent results. This has stimulated interest in the possibility of obtaining other natural control agents for use against citrus pests.

Photographed by Boyd George

# Screw-worm Program in Southwest

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An intensified program of screw-worm research was begun in 1960 in anticipation of a Southwestern Screw-worm Eradication Program which is now under way. Progress has been made in the following fields of research: (1) Radiation biology, (2) chemical sterilization, (3) attractants, (4) genetic markers, (5) nutrition, and (6) ecology. Some research findings have already been of direct benefit to the eradication program, and there is promise of further developments in efficiency and economy of entomological procedures. (Bushland. Jour. Econ. Ent. In Press.)

# Mass Rearing of Fruit Flies for Control and Eradication Programs

Norimitsu Tanaka, Entomologist
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Honolulu, Hawaii

An eradication program utilizing sterile melon flies, Dacus cucurbitae Coq., is being conducted on Rota Island in the Marianas, 40 miles north of Guam. Initially the experiment was against two species, the oriental fruit fly, Dacus dorsalis Hendel, and the melon fly. When the program was converted to the melon fly alone, production of this species was increased from one million to ten million pupae a week. Improvements in handling methods, efficient equipment, and substitution of cheaper materials have reduced the cost of rearing from $300 to $130 per million. This includes labor.

The method of rearing ten million melon fly pupae is described. This technique can be used with modification in producing the oriental fruit fly and the Mediterranean fruit fly, Ceratitis capitata Wied.

In order to mass produce the required number of pupae, eight to twelve cages are discarded and set up each week from a bank of forty. Each cage is stocked with 22,000 pupae from which approxmiately 20,000 flies emerge. The sex ratio is 1:1. The adult diet consists of Fleischmann's yeast hydrolyzate, sugar, and water. After eight days, the adults are gravid.

A plastic juice container with pin holes is used as an egging receptacle. A sponge in screen bag is soaked in diluted tomato juice and placed into each container. Eggs are collected by placing the container through a side opening of the cage and exposing it to the flies for 18-20 hours. The eggs are washed out of the containers into a cloth net and measured volumetrically into lots of 40,000 and seeded on six liters of medium. The larval medium is made up of dehydrated carrot-squash fortified with Brewer's yeast, sodium benzoate (mold inhibitor), and hydrochloric acid for adjusting the pH to 4.5 to 4.8.

After five days the third instar mature larvae are separated from the medium by washing through a sieve (16-mesh) under running water. One liter of washed larvae is mixed with six liters of moist vermiculite and allowed to pupate.

The pupae are separated from the vermiculite in a mechanical sifter. Pupae produced over a week are held at 68° and 80° F. for different lengths of time and adjusted so that the pupae will be seven days old at the time of irradiation. The irradiated pupae are then shipped via air parcel post to Guam and repackaged in release cartons and emergence cages. The flies emerge within 24-48 hours after receipt and are later distributed over Rota by aircraft (aerial release) and in emergence cages on the ground (ground release).

If the eradication procedure is feasible and larger production of flies is needed, the rearing technique can be mechanized to meet the increased demand.

## Closing Statement by Dr. Hoffmann

I know each of you realizes that this Conference means much to Dr. Knipling who regrets very much not being able to remain for all of the sessions. He was overwhelmed by the "Certificate of Merit" which was presented by those attending this Conference and I am sure that he will long cherish this unexpected award. The Division is making great strides in the control of insects, both by chemical and non-chemical means, and this progress is dependent upon the thought and effort that each of you as research leaders and workers put into the program. Incidentally, because of the interest of our Director in new approaches to insect control, he frequently raises questions bearing on the numbers of a particular insect per acre and the cost of rearing insects per thousand or million. Are you giving these matters some serious thought?

We all owe the program committee and the many who assisted with the various superb arrangements our hearty thanks. Our Discussion Leaders assisted materially in presenting a very comprehensive program in a well organized fashion. I also wish to congratulate each participant for the fine contributions made to the program and for actively taking part in the discussions. We have had a wonderful opportunity of becoming better acquainted personally and also with the many facets of research work in the Division. Moreover, I wish to thank each of you for the time and effort spent in preparing the excellent slides, charts, and films that have depicted your research work so well.

As a result of this working Conference, we all have a better appreciation of basic studies in insect nutrition and that such studies are the key to rearing insects required for screening insecticides; mass production of insect pathogens, parasites, and predators; studies on insect attractants; and the release of large numbers of insects for their own destruction following radiation or chemical sterilization. We have heard of ways in which to improve chemically defined or reproducible diets and our discussions have brought out many problems to be solved in connection with improved rearing techniques, particularly as they relate to methods of mass rearing. Examples of mass rearing of insects in laboratories and in large field cages have been cited and in some instances the costs are quite reasonable. Certainly the ground work has been laid at this Conference for making many improvements through further research and testing of insect diets and mass rearing procedures. As such information becomes available, it can be utilized in the development of new approaches for insect control by first making it possible for these to be proven experimentally in large cages and in isolated field tests before they are finally applied on a cooperative community basis.